SHOUT
SHARE
IT

THE

BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University

H. B. STEINBACH, Washington University
Managing Editor

VOLUME 85

AUGUST TO DECEMBER, 1943

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

The Biological Bulletin is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $1.75. Subscription per volume (three issues), $4.50.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Massachusetts, between July 1 and October 1, and to the Department of Zoology, Washington University, St. Louis, Missouri, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

LANCASTER PRESS, INC., LANCASTER, PA.

CONTENTS

No. 1. AUGUST, 1943

PAGE

ANNUAL REPORT OF THE MARINE BIOLOGICAL LABORATORY 1

SONNEBORN, T. M., AND RUTH V. DIPPELL
Sexual Isolation, Mating Types, and Sexual Responses to Diverse Conditions in Variety 4, Paramecium Aurelia 36

HOVANITZ, WILLIAM
Hybridization and Seasonal Segregation in Two Races of a Butterfly Occurring Together in Two Localities 44

LEVINE, HARRY P.
Species Differences in Rates of Osmotic Hemolysis Within the Genus Peromyscus 52

LAWSON, CHESTER A.
Germarial Differences and the Production of Aphid Types 60

LOOSANOFF, VICTOR L., AND JAMES B. ENGLE
Polydora in Oysters Suspended in Water 69

WAIT, ROBERT B.
The Action of Acetylcholine on the Isolated Heart of Venus Mercenaria 79

No. 2. OCTOBER, 1943

PYLE, ROBERT W.
The Histogenesis and Cyclic Phenomena of the Sinus Gland and X-Organ in Crustacea 87

BURT, AGNES SANXAY
Neurulation in Mechanically and Chemically Inhibited Amblystoma.. 103

PRATT, DAVID M.
Analysis of Population Development in Daphnia at Different Temperatures 116

HARVEY, ETHEL BROWNE
Rate of Breaking and Size of the "Halves" of the Arbacia Punctulata Egg when Centrifuged in Hypo- and Hypertonic Sea Water 141

HARVEY, ETHEL BROWNE, AND THOMAS F. ANDERSON
The Spermatozoon and Fertilization Membrane of Arbacia Punctulata as Shown by the Electron Microscope 151

BODINE, JOSEPH HALL, AND THEODORE NEWTON TAHMISIAN
The Development of an Enzyme (Tyrosinase) in the Parthenogenetic Egg of the Grasshopper, Melanoplus Differentialis 157

RIS, HANS
A Quantitative Study of Anaphase Movement in the Aphid Tamalia.. 164

56969

PAGE

No. 3. DECEMBER, 1943

HARRIS, DANIEL L.
The Osmotic Properties of Cytoplasmic Granules of the Sea Urchin Egg 179
WILBUR, KARL M., AND RICHARD O. RECKNAGEL
The Radiosensitivity of Eggs of Arbacia Punctulata in Various Salt Solutions 193
CLARKE, GEORGE L., E. LOWE PIERCE AND DEAN F. BUMPUS
The Distribution and Reproduction of Sagitta Elegans on Georges Bank in Relation to the Hydrographical Conditions 201
STUNKARD, HORACE W.
The Morphology and Life History of the Digenetic Trematode, Zoögonoides Laevis Linton, 1940 227
WHITING, P. W.
Intersexual Females and Intersexuality in Habrobracon 238
THIVY, FRANCESCA
New Records of Some Marine Chaetophoraceae and Chaetosphaeridiaceae for North America 244
HUGHES-SCHRADER, SALLY
Polarization, Kinetochore Movements, and Bivalent Structure in the Meiosis of Male Mantids 265

Vol. 85, No. 1 August, 1943

THE

BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

FORTY-FIFTH REPORT, FOR THE YEAR 1942—FIFTY-FIFTH YEAR

I. TRUSTEES AND EXECUTIVE COMMITTEE (AS OF AUGUST 11, 1942) 1
STANDING COMMITTEES 2
II. ACT OF INCORPORATION 3
III. BY-LAWS OF THE CORPORATION 4
IV. REPORT OF THE TREASURER 5
V. REPORT OF THE LIBRARIAN 9
VI. REPORT OF THE DIRECTOR 11
Statement 11

Addenda:

1. The Staff, 1942 13
2. Investigators and Students, 1942 16
3. Tabular View of Attendance 21
4. Subscribing and Co-operating Institutions, 1942 22
5. Evening Lectures, 1942 22
6. Shorter Scientific Papers, 1942 23
7. Members of the Corporation 24

I. TRUSTEES

EX OFFICIO

FRANK R. LILLIE, *President Emeritus of the Corporation*, The University of Chicago.
LAWRASON RIGGS, *President of the Corporation*, 120 Broadway, New York City.
E. NEWTON HARVEY, *Vice President of the Corporation*, Princeton University.
CHARLES PACKARD, *Director*, Marine Biological Laboratory.
OTTO C. GLASER, *Clerk of the Corporation*, Amherst College.
DONALD M. BRODIE, *Treasurer*, 522 Fifth Avenue, New York City.

EMERITUS

ROSS G. HARRISON, Yale University.
H. S. JENNINGS, University of California.
C. E. MCCLUNG, University of Pennsylvania.
S. O. MAST, Johns Hopkins University.
A. P. MATHEWS, University of Cincinnati.
T. H. MORGAN, California Institute of Technology.
W. J. V. OSTERHOUT, Rockefeller Institute.
G. H. PARKER, Harvard University.
W. B. SCOTT, Princeton University.

TO SERVE UNTIL 1946

Dugald E. S. Brown, New York University.
E. R. Clark, University of Pennsylvania.
Otto C. Glaser, Amherst College.
E. N. Harvey, Princeton University.
M. H. Jacobs, University of Pennsylvania.
F. P. Knowlton, Syracuse University.
Franz Schrader, Columbia University.
B. H. Willier, Johns Hopkins University.

TO SERVE UNTIL 1945

W. R. Amberson, University of Maryland School of Medicine.
S. C. Brooks, University of California.
W. C. Curtis, University of Missouri.
H. B. Goodrich, Wesleyan University.
I. F. Lewis, University of Virginia.
R. S. Lillie, The University of Chicago.
A. C. Redfield, Harvard University.
C. C. Speidel, University of Virginia.

TO SERVE UNTIL 1944

Eric G. Ball, Harvard University Medical School.
R. Chambers, Washington Square College, New York University.
Eugene F. DuBois, Cornell University Medical College.
W. E. Garrey, Vanderbilt University Medical School.
Columbus Iselin, Woods Hole Oceanographic Institution.
C. W. Metz, University of Pennsylvania.
H. H. Plough, Amherst College.
W. R. Taylor, University of Michigan.

TO SERVE UNTIL 1943

W. C. Allee, The University of Chicago.
G. H. A. Clowes, Lilly Research Laboratory.
B. M. Duggar, University of Wisconsin.
L. V. Heilbrunn, University of Pennsylvania.
Laurence Irving, Swarthmore College.
J. H. Northrop, Rockefeller Institute.
A. H. Sturtevant, California Institute of Technology.
Lorande L. Woodruff, Yale University.

Executive Committee of the Board of Trustees

Lawrason Riggs, *Ex officio, Chairman.*
E. N. Harvey, *Ex officio.*
D. M. Brodie, *Ex officio.*
Charles Packard, *Ex officio.*
D. E. S. Brown, to serve until 1943.
B. H. Willier, to serve until 1943.
C. W. Metz, to serve until 1944.
Otto C. Glaser, to serve until 1944.

The Library Committee

A. C. Redfield, *Chairman.*
E. G. Ball.
S. C. Brooks.
M. E. Krahl.
J. W. Mavor.

The Apparatus Committee

D. E. S. Brown, *Chairman.*
C. L. Claff.
G. Failla.
S. E. Hill.
A. K. Parpart.

The Supply Department Committee

L. G. Barth, *Chairman.*
E. G. Ball.
P. S. Galsoff.
R. T. Kempton.
D. A. Marsland.

The Evening Lecture Committee

B. H. Willier, *Chairman.*
M. H. Jacobs.
Charles Packard.

The Instruction Committee

H. B. Goodrich, *Chairman.*
W. C. Allee.
S. C. Brooks.
Viktor Hamburger.
Charles Packard.

II. ACT OF INCORPORATION

No. 3170

Commonwealth of Massachusetts

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, Henry B. Pierce, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11.30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two groups of Trustees as follows: (*a*) Trustees *ex officio*, who shall be the President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. Inasmuch as the time and place of the Annual Meeting of Members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of said meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

To The Trustees of the Marine Biological Laboratory:

Gentlemen:

Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1942.

The accounts have been audited by Messrs. Seamans, Stetson and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

The principal summaries of their report—The Balance Sheet, Statement of Income and Expense, and Current Surplus Account—are appended hereto as Exhibits A, B and C.

The following are some general statements and observations based on the detailed reports:

I. Assets

1. Endowment Assets

At the end of 1942 the total of all the Endowment Assets was $1,071,990.90, a loss of $7,821.17 from the preceding total, due largely to the loss of $8,801.13 incurred in the sale of one of the New York City real estate holdings on which the Laboratory had held a mortgage participation. The *market* value of the marketable securities increased slightly during the year. Using book values for the mortgage and real estate participations for which there are no market values, the total market value of all Endowment Assets was $1,020,282.41, compared with $999,599.86 at the end of 1941.

2. Plant Assets

The total of Plant Assets (excluding the Gansett and Devil's Lane Tracts) was $1,357,761.97 after deduction of $608,146.02 accumulated Depreciation Reserve. This represents a decrease of $18,407.84. Actual additions to Plant Assets during the year totalled $12,208.30 but this gain was more than offset by depreciation charges on buildings and equipment amounting to $26,935.14.

During the year $108.00 was expended on the construction of the Library addition. This left a balance of $2,762.07 remaining from the gifts totalling $110,400.00 received in 1940 and 1941 from the Rockefeller Foundation for the addition. This unexpended balance of $2,762.07 was returned to the Rockefeller Foundation in December in accordance with the understanding with the donor.

3. Current Assets

Current Assets, including cash, inventories and investments not in the Endowment Funds, amounted to $164,669.02, a decrease of $7,001.09. Current Liabilities (accounts payable) were $4,996.85 as compared with $6,423.47 so that Current Surplus was down only $5,574.47 to a total of $159,672.77.

II. Income and Expenditures

Total Income was $146,069.76, a decrease of $16,676.94 from 1941. Total expenditures including the $26,935.14 added to Depreciation Reserves were $163,281.69, a decrease of $8,947.02. The deficit for the year was, therefore, $17,211.93 as compared with the 1941 deficit of $9,482.01.

The decline in income was due to several factors. Income from the General Endowment and Library Funds was down from $38,879.73 in 1941 to $35,883.81. Dividends from the General Biological Supply House, Inc., dropped from $17,780.00 to $10,922.00. "Research" net income declined from $8,606.65 to $4,948.42. "Instruction" resulted in a net loss of $2,063.57 instead of the 1941 net profit of $176.45.

The Laboratory Administration met the problem created by reduced income by reducing operating expenses as shown in the detailed appendices. Maintenance expenses were substantially reduced and the usual deficits in operation of the mess, dormitory and supply departments (deficits caused only by depreciation and rental charges) were lessened. The rentals received from the United States Navy for the Laboratory properties under lease (Mess Hall, Apartment House, etc.) were also of assistance in reducing the deficit. Such rentals actually paid in 1942 amounted to $10,847.47 and were allocated to the respective accounts. In addition as of December 31st there were rental accruals due from the Navy of $1,677.47.

EXHIBIT A

Marine Biological Laboratory Balance Sheet, December 31, 1942

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee		$1,062,364.97	
Securities and Cash in Minor Funds		9,625.93	
			$1,071,990.90
Plant Assets:			
Land	$ 111,425.38		
Buildings	1,322,315.51		
Equipment	185,313.69		
Library	320,069.89		
	$1,939.124.47		
Less Reserve for Depreciation	608,146.02		
		$1,330,978.45	
Cash in Reserve Fund		4,273.51	
Cash in Book Fund		22,510.01	
			$1,357,761.97

Current Assets:			
Cash		$ 4,965.40	
Accounts Receivable		18,537.86	
Inventories:			
Supply Department	$ 31,683.18		
Biological Bulletin	12,768.29		
		$ 44,451.47	
Investments:			
Devil's Lane Property	$ 45,720.27		
Gansett Property	100.68		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Stocks	17,770.00		
Retirement Fund	14,137.88		
		$ 90,428.83	
Prepaid Insurance		4,291.72	
Items in Suspense		1,994.34	
			$ 164,669.62
Total Assets			$2,594,422.49

Liabilities

Endowment Funds:			
Endowment Funds	$1,060.069.32		
Reserve for Amortization of Bond Premiums	2,295.65		
		$1,062.364.97	
Minor Funds		9,625.93	
			$1,071,990.90
Plant Liabilities and Surplus:			
Donations and Gifts		$1,172,564.04	
Other Investments in Plant from Gifts and Current Funds		185,197.93	
			$1,357,761.97
Current Liabilities and Surplus:			
Accounts Payable		$ 4,996.85	
Current Surplus (Exhibit C)		159,672.77	
			$ 164,669.62
Total Liabilities			$2,594,422.49

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1942

Income:

	Total		Net	
	Expense	Income	Expense	Income
General Endowment Fund		$ 29,549.85		$ 29,549.85
Library Fund		6,333.96		6,333.96
Instruction	$ 7,508.57	5,445.00	$ 2,063.57	
Research	5,600.98	10,549.40		4,948.42

Evening Lectures	8.95		8.95	
Biological Bulletin and Membership Dues	7,575.89	8,244.44		668.55
Supply Department	42,306.45	40,607.40	1,699.05	
Mess	16,040.39	14,436.11	1,604.28	
Dormitories	21,151.71	12,676.29	8,475.42	
(Interest and Depreciation charged to above 3 Departments)	24,197.25			24,197.25
Dividends, General Biological Supply House, Inc.		10,922.00		10,922.00
Dividends, Crane Company		500.00		500.00
Rents:				
Bar Neck Property	648.80	4,338.02		3,690.12
Janitor House	24.16	360.00		335.84
Danchakoff Cottages	270.11	600.00		329.89
Lecture Hall and Botany Building		666.66		666.66
Sale of Library Duplicates and Micro Films		89.82		89.82
Apparatus Rental		689.63		689.63
Sundry Income		60.28		60.28
Maintenance of Plant:				
Buildings and Grounds	21,419.05		21,419.05	
Apparatus Department	5,952.23		5,952.23	
Chemical Department	2,789.77		2,789.77	
Library Expense	7,883.29		7,883.29	
Workmen's Compensation Insurance	541.87		541.87	
Truck Expense	307.04		307.04	
Bay Shore Property	86.57		86.57	
Great Cedar Swamp	19.35		19.35	
General Expenses:				
Administration Expense	12,501.53		12,501.53	
Endowment Fund Trustee and Safekeeping	1,014.45		1,014.45	
Bad Debts	355.78		355.78	
Special Repairs, Supply Dep't Stone Building	5,811.86		5,811.86	
Payment to former Technical Director	725.00		725.00	
Reserve for Depreciation	26,935.14		26,935.14	
	$163,281.69	$146,069.76	$100,194.20	$ 82,982.27
Excess of Expense over Income carried to Current Surplus		$ 17,211.93		$ 17,211.93
		$163,281.69		$100,194.20

EXHIBIT C

Marine Biological Laboratory, Current Surplus Account
Year Ended December 31, 1942

Balance, January 1, 1942		$165,247.24
Add:		
Reserve for Depreciation Charged to Plant Funds	$26,935.14	
Bad Debts Recovered	36.39	
Gain on Gansett Lot Sold	47.83	
		$ 27,019.36
		$192,266.60

Deduct:			
Excess of Expense over Income for Year as shown in Exhibit B..		$17,211.93	
Payments from Current Funds during Year for Plant Assets:			
Buildings	$ 3,192.99		
Equipment	1,580.98		
Library	7,449.33		
	$12,223.30		
Less Received for Plant Assets Disposed of	15.00		
		$12,208.30	
Pensions Paid	$ 3,460.00		
Less Retirement Fund Income	286.40		
		$ 3,173.60	
			$ 32,593.83
Balance, December 31, 1942			$159,672.77

Respectfully Submitted,

Donald M. Brodie,
Treasurer.

V. REPORT OF THE LIBRARIAN

The Library budget for 1942 was greatly reduced by action of the Executive Committee. For the years 1934–41 inclusive it was $18,850 per year, with only slight variations; for 1942 it was $12,200, a decrease of more than $6,000. Since 1940 we have received fewer and fewer European continental journals, until now practically none come in. Our subscriptions, however, are kept up, and the journals which cannot be delivered are being stored for the duration. Meanwhile no payments for these subscriptions have been made. For this reason there was an unexpended balance at the end of 1940. In 1940 the Library Committee requested that the balance, amounting to $3,977.18, be placed in a reserve fund from which to pay for the journals and back sets at such a time as they might be delivered. This request was granted by the Executive Committee. Early in the year 1941 a sum of $2,228.32 was so spent. A similar request in 1941 was not granted, and the unexpended balance of $2,663.48 for that year reverted to the general fund of the Laboratory. No request for a reserve fund was made in 1942. The Laboratory is now committed to pay for three years of foreign subscriptions, assuming that the journals can be delivered at some future time. There is now no adequate reserve fund from which such payments may be made.

This year the $12,200 appropriated was expended as follows: books, $91.06; serials, $1,489.28; binding, $1,084.05; express, $174.63; supplies, $471.15; salaries, $7,200; back sets, $1,797.92; sundries, $26.75; and insurance, $45.00; total, $12,379.84. The sales of duplicates brought in this year $26.06 and the income from the microfilm service inaugurated in the summer amounted to $63.76, the expenses for this latter having been charged to "supplies" and "salaries."

From the "Carnegie Fund" $2,239.01 was spent for back sets and journals and $250.98 for valuable books that we term biological "classics"; in all, 15 completed back sets, 24 partially completed and 23 "classics."

The Woods Hole Oceanographic Institution appropriated $800 to the Library for 1942 and a balance of $154.65 remained from the 1941 budget. An expended sum of $884.54 has been reported to the Director. A balance of $70.11 was carried on to the year 1943.

Since practically no current issues of journals have come to us from Europe since June 1941, it seems best in this report to give the figures for current journals actually received rather than for the subscriptions and exchange orders due us. This explains the sharp drop in this item that follows as compared with that for 1941. In 1942 the Library received 637 current publications (1,297 in 1941): 227 (11 new) in subscriptions to the Marine Biological Laboratory, 18 (1 new) to the Woods Hole Oceanographic Institution; 209 exchanges, 192 (2 new) with the "Biological Bulletin" and 17 (0 new) with the Woods Hole Oceanographic Institution publications; 178 gifts to the former and 5 to the latter. The Marine Biological Laboratory acquired 107 books; 43 by purchase of the Marine Biological Laboratory (23 "classics" *see above*), 15 by purchase of the Woods Hole Oceanographic Institution; 16 as gifts from the authors, 30 from publishers and 3 miscellaneous. There were 47 back sets of serial publications completed; 34 purchased by the Marine Biological Laboratory (15 with "Carnegie Fund"); 2 by the Woods Hole Oceanographic Institution; 1 secured by exchange of the "Biological Bulletin"; 5 as gifts to the same and 5 by exchange of duplicate material. Partially completed sets were 164: purchased by the Marine Biological Laboratory, 59 (24 with "Carnegie Fund"); by the Woods Hole Oceanographic Institution, 3; by exchange of the "Biological Bulletin," 1; by gift to the same, 44; and by exchange of duplicate material, 57. The reprint additions number 3,097: current of 1941, 436; current of 1942, 23; and of previous dates, 2,638. The present holdings of the Library are 50,937 bound volumes and 122,723 reprints.

Very few of the current reprints received were catalogued during 1942. From May until November three members of the staff spent the major part of their working hours on the "List of serial holdings" to be published as a "supplement" to the "Biological Bulletin" in the February 1943 issue. The total reprints of date 1941 therefore will be recorded, as well as those of the date 1942, in the 1943 report. The current reprints separated from those of previous dates were first counted in the 1937 report and are summarized as follows: 1936–37, 4,602; 1938, 2,453; 1939, 2,246; and 1940, 1,887. The decline in current reprints in 1940 continues in 1941–42. It would seem that the efforts made so far by the Librarian to impress upon investigators the importance of these current reprints can have had no sustained effect. The best results were obtained by personal interviews of the Library with individuals and credit must be given to those, and they are considerable in number, who do conscientiously send their reprints as issued. Perhaps a better method of keeping the collection to date may be devised when the war conditions are over.

During the year seven valuable gifts in non-current reprints were received; a total of 17,017. Of these 7,759 were new to us and will be filed for use; 9,258, being duplicate, will be placed in our duplicate files and any third copies will be for sale. The Library is indebted to Dr. Rudolf Höber for the generous gift of his collection of 7,217 reprints in the subjects of physical and physiological chemistry and physiology; to Dr. H. E. Crampton, for the high figure of 5,102 reprints on miscellaneous subjects; from Mrs. H. J. Fry and Dr. Robert Chambers, Dr. Fry's collection in cytology, 2,660 in all; Dr. D. J. Edwards contributed 606 reprints; Dr. E. J. Herrick, 383; Dr. Libbie H. Hyman, 965; and Dr. B. M. Davis, 84. Miss Mathilda Koch kindly sent to us several sets of journals and four books from the Library of her brother, Dr. Waldemar Koch, with the understanding that the books should be incorporated in the Library, and the journals, which are duplicates to us, should revert to our use for sale or exchange in case the sale of these is not consummated within a given period. The addition of 7,759 reprints to the back files of reprints is the highest number that has ever been added in one year to the Library's collection and Dr. Höber's gift is the largest single collection that this Library has ever received.

VI. THE REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory:
Gentlemen:

I beg to submit herewith a report of the fifty-fifth session of the Marine Biological Laboratory for the year 1942.

1. *Changes in Personnel.* At the Trustees' meeting in 1940, Dr. F. R. Lillie presented his resignation as President of the Corporation and Chairman of the Executive Committee. He was persuaded, however, to continue his duties until suitable preparations could be made for naming his successor. The Committee entrusted with this responsibility agreed that the office of President deals largely with the external relations of the Laboratory and that search should be made for some one who would appropriately represent the Laboratory in this field. At the same time they felt that there should be a Vice President who would represent Biology. After due consideration, Lawrason Riggs, Treasurer of the Corporation since 1924, was nominated for the Presidency, and Dr. E. Newton Harvey was named to fill the newly created position of Vice President. They were formally elected to these offices at the Trustees' meeting in 1942. At the Corporation meeting, Mr. Donald M. Brodie, formerly manager of Mr. C. R. Crane's New York office, was elected Treasurer in place of Mr. Riggs, and Dr. Otto Glaser was elected Clerk of the Corporation in place of Dr. P. B. Armstrong who resigned because of pressure of war work. The Laboratory is fortunate in securing the services of these men and confidently gives them its whole-hearted support. Dr. Lillie was named President Emeritus of the Corporation. Dr. Packard, the Director, was made Resident Director, and assumed his full time duties at the Laboratory on October 1, 1942.

2. *Financial.* At the present time the financial condition of the Laboratory is satisfactory, even though our income has fallen during the past few years. In 1942 it was about 16 per cent below the average of the preceding eight years, the decrease being due, in large measure, to a sharp drop in returns from the endowment funds and from dividends, from research fees, and from the courses of instruction. It is a matter of gratification that many of our subscribing institutions have continued their support even though they can send few representatives or none at all. On the other hand, the income from the Supply Department increased, and so also did the item of Rentals, a result of the Navy's occupation of the Apartment House, the Mess, and other buildings. At the same time our expenditures have been reduced. The cost of maintaining the buildings, and of administration,

has fallen somewhat below the average, but the chief reductions are in the appropriations for the Library and the Apparatus Department. These economies have, in a sense, been forced upon us. A large proportion of the foreign journals, to which we are still subscribing, can no longer be delivered to us; our payments for them have therefore ceased. Then, also, we can buy little new scientific equipment. Thus far this has not worked any great hardship on the investigators, for with reduced attendance, the call for apparatus has lessened. What we have on hand can be adapted to new needs with the aid of the Apparatus Department staff. Under these conditions the Laboratory can continue to operate within its budget.

But these conditions will not long continue. When normal interchange with Europe is re-established we shall presumably receive the journals now held in storage for us, and for them we must pay approximately the amount which was taken from the Library appropriation. So, also, after the war we shall need a substantial sum for the replacement of old apparatus, and more particularly, for the purchase of new tools for research to be used in new fields, such as electronics, which have been so greatly developed within the last few years.

3. *Attendance.* A comparison of the attendance at the Laboratory in 1941 and 1942 with the average of the preceding five-year period shows how seriously the war is affecting us. The years 1936–1940 marked the highest attendance in our history. In 1937 the total registration was 511, and in 1940, 507. The decline began in 1941 when the number of independent investigators fell off noticeably. The other groups, however, were present in normal numbers. In 1942 the attendance in all groups declined sharply, the change being most marked among the younger members. Only about one-third of the usual number of assistants was present, and only one-fourth of the beginning investigators. In the classes, attendance dropped to about two-thirds of the average except in Physiology where the falling off was greater. Many of the investigators taught at their colleges throughout the summer, and will continue to do so for the duration. Others are engaged on wartime problems which they are carrying on at their own institutions. In many cases this research is in their chosen field, so their time is by no means lost. Indeed in some instances it has already opened up new fields for future exploration. But the armed services have absorbed a large proportion of the younger generation who normally would take our courses or begin research. For some time to come, few young and vigorous minds will be added to our list of investigators. All the more it is encumbent upon those who remain to continue and to extend their peacetime investigations.

RECORD OF ATTENDANCE, 1936–1942

	1936	*1937*	*1938*	*1939*	*1940*	*Ave.*	*1941*	*1942*
Independent Investigators	226	256	246	213	253	239	228	160
Assistants	57	61	81	79	71	70	50	25
Beginning Investigators	76	74	53	60	62	65	59	16
Students	138	133	132	133	128	133	131	74
Corrected Totals	473	511	496	471	507	489	461	273

4. *Losses by Death.* In the death of Dr. Calkins the Laboratory loses a devoted friend. His important services as Clerk of the Corporation, Secretary of the Board of Trustees, as an active member of many committees, and as head of the Protozoology course, will long be remembered.

During the year also occurred the death of one of our Life Members, Dr. A. Lawrence Lowell, who served as Clerk of the Corporation from 1890 to 1894.

5. *The Stone Building.* During the fall and winter the Stone Building has been completely renovated. The decision to do this was made when the Executive Committee, after a tour of inspection, realized how serious the condition was. Only a part of the basement could be used; the stairs were no longer safe; the first and second floors were not strong enough to permit the storage of heavy tanks; the shingles and trim were in bad shape. These deficiencies have been corrected. The entire basement is now available for storage, there is a new concrete floor, a new heating system, and adequate plumbing and lighting. To provide more head room, the ceiling was raised 18 inches. Many steel columns, both in the basement and in the first floor, support the great carrying beams which are still sound. The front of the first floor is now divided into offices and laboratories. The business of the Supply Department can therefore be carried on in the Stone building, leaving the wooden building to be used primarily for the preparation of material. These changes were planned and carried out by Mr. Larkin and Mr. Hemenway, the latter bearing the larger share of the work. Both the inside and the outside of the building are now in excellent condition.

In summary, the Laboratory during these disturbing times is maintaining its usual services, and by rigid economies, is balancing its budget. This quiescent period will not long continue; we must prepare for an expansion of our research facilities soon after the war ends.

6. *Election of Trustees.* At the meeting of the Corporation held August 11, 1942, the following Trustees were elected Trustees Emeritus:

A. P. Mathews, University of Cincinnati
S. O. Mast, The Johns Hopkins University

The new Trustees elected at that meeting are:

Eugene F. DuBois, Class of 1944
Eric G. Ball, Class of 1944

7. There are appended as parts of this report:

1. The Staff.
2. Investigators and Students.
3. Tabular View of Attendance, 1938–1942.
4. Subscribing and Co-operating Institutions.
5. Evening Lectures.
6. Shorter Scientific Papers.
7. Members of the Corporation.

Respectfully submitted,

CHARLES PACKARD,
Director

1. THE STAFF, 1942

CHARLES PACKARD, Director, Marine Biological Laboratory, Woods Hole, Massachusetts.

SENIOR STAFF OF INVESTIGATION

GARY N. CALKINS, Professor Emeritus of Protozoology, in residence, Columbia University.

E. G. Conklin, Professor of Zoology, Emeritus, Princeton University.
Caswell Grave, Professor of Zoology, Emeritus, Washington University.
Frank R. Lillie, Professor of Embryology, Emeritus, The University of Chicago.
Ralph S. Lillie, Professor of General Physiology, The University of Chicago.
C. E. McClung, Professor of Zoology, Emeritus, University of Pennsylvania.
S. O. Mast, Professor of Zoology, Johns Hopkins University.
A. P. Mathews, Professor Emeritus, Biochemistry, University of Cincinnati.
T. H. Morgan, Director of the Biological Laboratory, California Institute of Technology.
G. H. Parker, Professor of Zoology, Emeritus, Harvard University.

ZOOLOGY

I. Consultants

T. H. Bissonnette, Professor of Biology, Trinity College.
L. L. Woodruff, Professor of Protozoology, Yale University.

II. Instructors

A. J. Waterman, Associate Professor of Biology, Williams College, in charge of course.
John B. Buck, Assistant Professor of Zoology, University of Rochester.
M. D. Burkenroad, Assistant Curator, Bingham Oceanographic Foundation, Yale University.
W. G. Hewatt, Professor of Biology, Texas Christian University.
W. E. Martin, Associate Professor of Zoology, DePauw University.
N. T. Mattox, Assistant Professor of Zoology, Miami University.
R. W. Wilhelmi, Instructor in Zoology, University of Missouri.

III. Laboratory Assistant

Ruth Merwin, University of Chicago.

EMBRYOLOGY

I. Consultants

L. G. Barth, Assistant Professor of Zoology, Columbia University.
H. B. Goodrich, Professor of Biology, Wesleyan University.

II. Instructors

Viktor Hamburger, Professor of Zoology, Washington University, in charge of course.
Donald P. Costello, Assistant Professor of Zoology, University of North Carolina (absent in 1942).
Charles B. Metz, Teaching Fellow, California Institute of Technology.
Olin Rulon, Assistant Professor of Biology, Wayne University.
Ray L. Watterson, Instructor in Embryology, Dartmouth College.

PHYSIOLOGY

I. Consultants

William R. Amberson, Professor of Physiology, University of Maryland, School of Medicine.
Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Merkel H. Jacobs, Professor of Physiology, University of Pennsylvania.

II. Instructors

Rudolf T. Kempton, Professor of Zoology, Vassar College, in charge of course.
Kenneth C. Fisher, Assistant Professor of Experimental Biology, University of Toronto.
Arthur C. Giese, Associate Professor of Biology, Stanford University.
F. J. M. Sichel, Assistant Professor of Physiology, University of Vermont, College of Medicine.

BOTANY

I. Consultants

S. C. Brooks, Professor of Zoology, University of California.
D. R. Goddard, Assistant Professor of Botany, University of Rochester.

II. Instructors

Wm. Randolph Taylor, Professor of Botany, University of Michigan, in charge of course.
Hannah Croasdale, Technical Assistant, Dartmouth College.

EXPERIMENTAL RADIOLOGY

G. Failla, Memorial Hospital, New York City.
L. Robinson Hyde, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian
Deborah Lawrence
Mary A. Rohan
S. Mabell Thombs

APPARATUS DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager
J. D. Graham
R. S. Liljestrand

CHEMICAL DEPARTMENT

Kenneth C. Ballard, Lawrence High School, Falmouth, Mass., Manager

SUPPLY DEPARTMENT

James McInnis, Manager
Ruth Crowell
M. B. Gray
A. M. Hilton
W. E. Kahler
A. W. Leathers
Grace Harman
G. Lehy
F. N. Whitman

GENERAL OFFICE

F. M. MacNaught, Business Manager
Polly L. Crowell
Glade F. Allen

GENERAL MAINTENANCE

T. E. Larkin, Superintendent
F. A. Cannon
W. C. Hemenway
R. W. Kahler
T. E. Tawell
R. F. Travis
J. Wynne

THE GEORGE M. GRAY MUSEUM

George M. Gray, Curator Emeritus

2. INVESTIGATORS AND STUDENTS

Independent Investigators, 1942

Addison, William H. F., Professor of Normal Histology and Embryology, University of Pennsylvania, School of Medicine.

Anderson, Thomas F., RCA Fellow, National Research Council.

Andrew, Warren, Assistant-Professor of Histology and Embryology, Baylor University, College of Medicine.

Baker, Horace B., Professor of Zoology, University of Pennsylvania.

Ball, Eric G., Associate Professor, Department of Biological Chemistry, Harvard Medical School.

Ball, Ernest, National Research Fellow in Botany, Yale University.

Barth, L. G., Assistant Professor of Zoology, Columbia University.

Bartlett, James H., Jr., Associate Professor of Theoretical Physics, University of Illinois.

Berger, Charles A., Professor of Cytology and Genetics, Fordham University.

Bissonnette, T. H., Professor of Biology, Trinity College.

Blum, John L., Instructor in Biology, Canisius College.

Bodian, David, Associate in Epidemiology, Johns Hopkins University.

Botsford, E. Frances, Associate Professor of Zoology, Connecticut College.

Brooks, Matilda M., Research Associate, University of California.

Brooks, Sumner C., Professor of Zoology, University of California.

Buck, John B., Assistant Professor of Zoology, University of Rochester.

Budington, R. A., Professor of Zoology, Emeritus, Oberlin College.

Burkenroad, Martin D., Assistant Curator, Peabody Museum, Yale University.

Cannan, R. Keith, Professor, New York University College of Medicine.

Chambers, Robert, Research Professor of Biology, Washington Square College, New York University

Cheney, Ralph H., Professor of Biology, Long Island University.

Child, Ruth C., Assistant Professor, Wellesley College.

Clark, Eleanor L., Department of Anatomy, University of Pennsylvania.

Clark, Eliot R., Professor and Director of Department of Anatomy, University of Pennsylvania, School of Medicine.

Clowes, G. H. A., Director of Research, Eli Lilly and Company.

Conklin, Edwin G., Professor of Biology, Emeritus, Princeton University.

Copeland, Manton, Professor of Biology, Bowdoin College.

Croasdale, Hannah T., Technical Assistant, Dartmouth College.

Delbruck, Max, Instructor in Physics, Vanderbilt University.

Dreyer, Nicholas B., Associate Professor of Pharmacology, Long Island College of Medicine.

Eakin, Richard M., Assistant Professor of Zoology, University of California.

Elizabeth, Sister Miriam, Associate Professor of Biology, Chestnut Hill College.

Evans, Titus C., Research Assistant Professor of Radiology, State University of Iowa.

Failla, G., Physicist, Memorial Hospital.

Fisher, Kenneth C., Assistant Professor of Physiological Zoology, University of Toronto.

Frey, David G., Junior Aquatic Biologist, U. S. Fish and Wildlife Service.

Frisch, John A., Professor of Biology, Head of Biology Department, Canisius College.

Gabriel, Mordecai L., Lecturer in Zoology, Columbia University.

Galtsoff, Paul S., Biologist in Charge Shellfish Investigation, U. S. Fish and Wildlife Service.

Garrey, W. E., Professor of Physiology, Vanderbilt University School of Medicine.

Giese, Arthur C., Associate Professor of Biology, Stanford University.

Glaser, Otto C., Professor of Biology, Amherst College.

Grand, C. G., Research Associate, Washington Square College, New York University.

Grave, Caswell, Professor of Zoology, Emeritus, Washington University.

Gurewich, Vladimir, Clinical Assistant and Attending Physician, Cornell Division of the Bellevue Hospital.

Hamburger, Viktor, Professor of Zoology, Washington University.

Hartman, Frank A., Professor and Chairman Department of Physiology, Ohio State University.
Harvey, Ethel B., Research Investigator, Princeton University.
Harvey, E. Newton, Professor of Physiology, Princeton University.
Haugaard, G., Research Assistant, Harvard University.
Haywood, Charlotte, Professor of Physiology, Mount Holyoke College.
Heilbrunn, L. V., Associate Professor of Zoology, University of Pennsylvania.
Henry, Richard J., Medical Student, School of Medicine, University of Pennsylvania.
Hewatt, Willis G., Professor of Biology, Texas Christian University.
Hill, Samuel E., Professor of Biology, Russell Sage College.
Hopkins, Hoyt S., Associate Professor of Physiology, New York University College of Dentistry.
Howe, H. E., Editor, Industrial and Engineering Chemistry.
Hyman, Chester, Research Assistant, New York University.
Jacobs, M. H., Professor of General Physiology, University of Pennsylvania Medical School.
Johlin, J. M., Associate Professor, Vanderbilt University School of Medicine.
Kempton, Rudolf T., Professor of Zoology, Vassar College.
Knowlton, Frank P., Professor of Physiology, Syracuse University, College of Medicine.
Kopac, M. J., Visiting Assistant Professor of Biology, New York University.
Krahl, M. E., Research Chemist, Eli Lilly and Company.
Lillie, Frank R., Professor of Embryology, Emeritus, The University of Chicago.
Lillie, Ralph S., Professor of Physiology, Emeritus, The University of Chicago.
Little, Elbert P., Instructor in Science, Phillips Exeter Academy.
Lowenstein, B. E., Research Associate, New York University, Washington Square College.
Luria, Salvador E., Research Assistant in Surgical Bacteriology, Columbia University.
McBride, Arthur F., Curator, Marine Studios Inc.
McClung, C. E., Professor of Zoology, Emeritus, University of Pennsylvania.
Marsland, Douglas A., Assistant Professor of Biology, Washington Square College, New York University.
Martin, Walter E., Associate Professor of Zoology, DePauw University.
Mast, S. O., Professor of Zoology, Johns Hopkins University.
Mathews, A. P., Professor of Biochemistry, Emeritus, University of Cincinnati.
Mattox, N. T., Assistant Professor of Zoology, Miami University.
Mavor, James W., Professor of Biology, Union College.
Memhard, Allen R., Crescent Rd., Riverside, Connecticut.
Menkin, Valy, Assistant Professor of Pathology, Harvard Medical School.
Metz, Charles W., Head, Department of Zoology, University of Pennsylvania.
Molter, John A., Graduate Student, University of Pennsylvania.
Moog, Florence, Graduate Student, Columbia University.
Morgan, T. H., Professor of Biology, California Institute of Technology.
Nabrit, S. Milton, Professor of Biology, Atlanta University.
Nachmansohn, David, Research Associate, Columbia University.
O'Brien, John A., Instructor in Biology, Catholic University of America.
Osterhout, W. J. V., Member Emeritus, Rockefeller Institute for Medical Research.
Packard, Charles, Director, Marine Biological Laboratory.
Pierson, Bernice F., Instructor in Biology, National Park College.
Plough, Harold H., Professor of Biology, Amherst College.
Pollister, Arthur W., Associate Professor of Zoology, Columbia University.
Pomerat, Gerard R., Instructor in Biology, Harvard University.
Richards, A. Glenn, Jr., Instructor in Zoology, University of Pennsylvania.
Ris, Hans, Zoology Department, Columbia University.
Rugh, Roberts, Associate Professor, Washington Square College, New York University.
Rulon, Olin, Assistant Professor, Wayne University.
Runyon, Ernest H., Associate Professor of Botany, Agnes Scott College.
Schallek, William B., Biological Laboratories, Harvard University.
Schaeffer, A. A., Professor and Chairman of the Department of Biology, Temple University.

Scott, Allan C., Assistant Professor of Biology, Union College.
Scott, Sister Florence M., Professor of Zoology, Seton Hill College.
Shanes, Abraham M., Instructor in Physiology, New York University, College of Dentistry.
Shaw, Myrtle, Senior Bacteriologist, New York State Department of Health.
Shelden, Frederick F., Instructor in Physiology, Ohio State University.
Sichel, Elsa Keil, Head of the Science Department, Vermont State Normal School.
Sichel, F. J. M., Assistant Professor of Physiology, University of Vermont, College of Medicine.
Simpson, Jennie L. S., Assistant Professor of Botany, Hunter College.
Slifer, Eleanor H., Assistant Professor, Department of Zoology, State University of Iowa.
Smelser, George K., Assistant Professor of Anatomy, Columbia University College of Physicians and Surgeons.
Springer, Stewart, Marine Studios, Inc.
Steinbach, H. B., Associate Professor of Zoology, Washington University.
Stewart, Dorothy R., Associate Professor of Biology, Skidmore College.
Stokey, Alma G., Professor Emeritus, Mount Holyoke College.
Stunkard, Horace W., Professor of Biology, New York University.
Taylor, William R., Professor of Botany, University of Michigan.
TeWinkel, Lois E., Assistant Professor of Zoology, Smith College.
Thivy, Francesca, Graduate Student, University of Michigan.
Trinkaus, J. Philip, Graduate Student, Johns Hopkins University.
Turner, Abby H., Professor of Physiology, Emeritus, Mount Holyoke College.
von Sallmann, Ludwig J., Assistant Professor in Ophthalmology, College of Physicians and Surgeons, Columbia University.
Waterman, Allyn J., Associate Professor of Biology, Williams College.
Wenrich, D. H., Professor of Zoology, University of Pennsylvania.
Wenstrup, Edward J., Head, Department of Biology, St. Vincent College.
Whiting, P. W., Associate Professor of Zoology, University of Pennsylvania.
Wiercinski, Floyd J., Research Assistant, University of Pennsylvania.
Wilbur, Karl M., Instructor, Ohio State University.
Wilhelmi, Raymond W., Instructor in Zoology, University of Missouri.
Willier, B. H., Professor of Zoology, The Johns Hopkins University.
Wolf, E. Alfred, Associate Professor of Biology, University of Pittsburgh.
Woodruff, Lorande L., Professor of Protozoology and Director of the Osborn Zoological Laboratory, Yale University.
Wrinch, Dorothy, Visiting Professor, Smith, Amherst and Mt. Holyoke Colleges.
Zweifach, Benjamin W., Research Associate in Biology, New York University.

Beginning Investigators

Brummer, Donald L., Student, New York University, College of Medicine.
Clark, Arnold M., Graduate Student, University of Pennsylvania.
Cole, Edith, Undergraduate Assistant, Pennsylvania College for Women.
Daniel, Sister Paul, Instructor, Chestnut Hill College.
Ferguson, Frederick P., Teaching Assistant, University of Minnesota.
Grosch, Daniel S., Assistant Instructor, University of Pennsylvania.
Hinchey, M. Catherine, Instructor in Biology, Temple University.
Jaeger, Lucena, Graduate Student, Columbia University.
Keltch, Anna K., Research Chemist, Eli Lilly and Co.
LeFevre, Paul G., Research Assistant, University of Pennsylvania.
Metz, Charles B., Teaching Fellow, California Institute of Technology.
Nelson, Leonard, Student, University of Pennsylvania.
Southwick, Mildred D., Instructor of Plant Science, Vassar College.
Taylor, Harriett E., Graduate Assistant, Columbia University.
Watterson, Ray L., Instructor, Dartmouth College.
Wilson, Walter L., Graduate Student, University of Pennsylvania.

Research Assistants

Atkinson, Lenette R., Research Assistant, Amherst College.
Barber, Ava J., Senior Student, University of California.
Bond, Christiana, Secretary, University of Maryland Medical School.
Brownell, Katharine A., Research Associate, Ohio State University.
Butler, Mary K., Research Assistant, University of Pennsylvania.
Cook, Elizabeth J., Research Assistant, Harvard University.
Dytche, Maryon M., Graduate Assistant, University of Pittsburgh.
Ehrenfeld, Klara, Research Assistant, Amherst College.
Garzoli, Ray F., Graduate Student, University of California.
Heidenthal, Gertrude, Research Assistant, University of Pennsylvania.
Hohwieler, Harold J., Graduate Assistant, Washington University.
Jacobs, Joye E., Research Assistant, University of Maryland Medical School.
Kibrick, Andre C., Teaching Assistant, New York University Medical College.
Kielich, E. Randolph, Graduate Assistant, Canisius College.
Krugelis, Edith J., Research Assistant, Columbia University.
Long, M. Jeanne, Research Assistant, New York University.
Machado, Angelo L., Research Fellow, Yale University Medical School.
Merritt, Frances A., Laboratory Assistant, Eli Lilly & Co.
Phillips, Clyde, Assistant in Anatomy, Morehouse College.
Smith, Douglas E., Research Assistant, Ohio State University.
Spiegelman, S., Research Assistant, Washington University.
Stevens, Hazel A., Laboratory Assistant, Eli Lilly and Co.
Stevens, Katharine, Student, Vassar College.
Woodward, Arthur A., Jr., Research Assistant, Wesleyan University.
Wurtz, Charles B., Graduate Student Assistant, University of Pittsburgh.

Library Readers, 1942

Amberson, William R., Professor of Physiology, University of Maryland Medical School.
Beck, L. V., Instructor in Physiology, Hahnemann Medical College.
Belda, Walter H., Assistant Professor, Fordham University.
Bloch, Robert, Research Assistant, Yale University.
Cassidy, Harold G., Yale University.
Clark, Helen, Instructor in Zoology, Hunter College of the City of New York.
Diamond, Louis K., Associate in Pediatrics, Harvard Medical School.
Diamond, Moses, Associate Professor, Columbia University Dental School.
Everett, Guy M., Weaver Research Fellow, University of Maryland Medical School.
Fowler, Coleen, Johns Hopkins University.
Gates, R. R., Professor, University of London.
Hutchings, Lois M., Teacher of Biology, Weequahic High School.
Jones, Arthur W., Research Fellow in Zoology, University of Virginia.
Kreezer, George L., Assistant Professor of Psychology, Cornell University.
Lavin, George, Rockefeller Institute for Medical Research.
Levine, Philip, Bacteriologist and Serologist, Beth Israel Hospital.
Loewi, Otto, Research Professor, New York University College of Medicine.
Ludwig, Francis W., Instructor, Villanova College.
Meyerhof, N. Otto, Research Professor of Biochemistry, University of Pennsylvania.
Mitchell, Philip H., Professor of Biology, Brown University.
Newell, James W., Student, Cornell University Medical College.
Oster, Robert H., Assistant Professor of Physiology, University of Maryland Medical School.
Renshaw, Birdsey, Assistant Professor, Oberlin College.
Roberts, Edith, Chairman, Department of Botany, Vassar College.
Sevag, M. G., Assistant Professor of Biochemistry, University of Pennsylvania School of Medicine.
Shapiro, Herbert, Instructor in Physiology, Hahnemann Medical College.
Shwartzman, Gregory, Head of Department of Bacteriology, The Mount Sinai Hospital.
Stiles, Karl A., Professor of Biology, Coe College.

Students, 1942

BOTANY

Arrowsmith, Harold N., Jr., Student, Johns Hopkins University.
Behnke, Jane, Student, Wellesley College.
Booth, Mary L., Student, Smith College.
Hitchcock, Margaret V., Goucher College.
Kingsley, Eunice L., Assistant Prof. of Botany, Kansas State College.
Paull, John J., Student, Washington and Jefferson College.
Richardson, Edward A., Graduate Assistant, Rutgers University.
Young, Margaret E., Assistant in Botany, Wellesley College.

EMBRYOLOGY

Beardsley, Margaret, Smith College.
Boss, Mary B., Goucher College.
Buggs, Charles W., Prof. of Biology and Head, Division of the Sciences, Dillard University.
Carpenter, Elizabeth, Graduate Assistant, Mount Holyoke College.
Churchill, Warren S., Assistant in Zoology, University of Illinois.
Cole, Edith, Undergraduate Assistant, Pennsylvania College for Women.
Dodd, Samuel G., Wesleyan University.
Dunn, Barbara, Graduate Assistant, Wellesley College.
Elias, Catherine, Volunteer Laboratory Assistant, Connecticut College.
Foster, James J., Graduate Assistant, Amherst College.
Gajdusek, D. Carleton, Student, University of Rochester.
Geisler, Sister Francis S., S.S.J., Student, Catholic University.
Littrell, June L., Assistant, University of Illinois.
Memhard, Allen R., Crescent Road, Riverside, Conn.
Newfang, Dorothy, Mount Holyoke College.
Nickerson, Mark, Graduate Assistant, Johns Hopkins University.
Philbrick, Madeline G., Russell Sage College.
Poindexter, Joan, Smith College.
Prodell, John H., Brothers College of Drew University.
Reyer, Randall W., Cornell University.
Seitner, Margaret M., Hunter College of the City of New York.
Senyard, Juanita, Graduate Assistant, Mount Holyoke College.
Shea, Samuel E., Jr., Student Laboratory Instructor, Canisius College.
Wood, Marcia, Student, Russell Sage College.

PHYSIOLOGY

Christiansen, Gertrude M., Assistant, Wellesley College.
Hardenbergh, Esther, Student, Mount Holyoke College.
Larson, Virginia P., Assistant in Physiology, Vassar College.
Low, Eva M., Student, Radcliffe College.
Osterman, George B., Instructor, Washington and Jefferson College.
Poker, Nathan, Brooklyn College.

ZOOLOGY

Avila, Enrique, Compania Administradora del Guano, Lima, Peru.
Benson, John A., Undergraduate Assistant, Wesleyan University.
Brearley, Margery, Graduate Student, Mount Holyoke College.
Chroniak, Walter, Massachusetts State College.
Cole, Elsie L., Heidelberg College.
Cole, M. Ethel, Teacher, Frick Educational Commission.
Collard, LaVerne E., Oberlin College.
Cosby, Evelyn L., Laboratory Instructor in Botany, University of Richmond.

Cregar, Mary, Wilson College.
Daughaday, Eleanor F., Vassar College.
Dintiman, Sara Mae, Rutgers University.
Donaldson, Sara L., Graduate Assistant, Syracuse University.
Doochin, Herman D., Student, University of Miami.
Fogg, N. W., Student, American International College.
Foster, James J., Graduate Assistant, Amherst College.
Franklin, Rev. Roger G., Prof. of Biology, St. Joseph's Seminary.
Haas, Elizabeth, Bennington College.
Hufford, Virginia, Oberlin College.
Hyde, Jane E., Student, Radcliffe College.
Johnson, Vieno T., 44 Francis Ave., Cambridge, Mass.
Keister, Margaret L., Instructor, Wheaton College.
Lesage, Maurice C., Teacher, Society of Divine Word.
Lorentz, John J., Graduate Student, Fordham University.
Manny, Ella T., Sarah Lawrence College.
Newcomer, Stanley, Assistant, Cornell University.
O'Rourk, Ann E., Duke University.
Peterson, Harold L., Student Assistant, Drew University.
Philbrick, Madeline G., Student, Russell Sage College.
Rayner, Harriet A., Massachusetts State College.
Saunders, John W., Graduate Assistant, Johns Hopkins University.
Schmeisser, Elizabeth F., Student, Sweet Briar College.
Taft, Edith D., Wheaton College.
Waterman, George E., Professor of Biology, Assumption College.
Weckstein, Abraham M., Instructor of Biology, New York University.
White, Marcia R., Student, Cornell University.
Wood, Marcia, Student, Russell Sage College.

3. TABULAR VIEW OF ATTENDANCE

	1938	*1939*	*1940*	*1941*	*1942*
Investigators—Total	380	352	386	337	201
Independent	246	213	253	197	132
Under instruction	53	60	62	59	16
Research assistants	81	79	71	50	25
Library readers				31	28
Students—Total	132	133	128	131	74
Zoology	54	55	55	55	36
Protozoology (not given after 1940)	10	12	7	—	—
Embryology	34	36	34	37	24
Physiology	22	21	22	24	6
Botany	12	9	10	15	8
Total Attendance	512	485	514	468	275
Less persons registered as both students and investigators	16	14	7	7	2
	496	471	507	461	273
Institutions Represented—Total	151	162	148	144	126
By investigators	125	132	112	102	83
By students	67	72	79	72	43
Schools and Academies Represented					
By investigators	4	2	1	5	2
By students	1	2	2	2	0
Foreign Institutions Represented					
By investigators	14	8	2	3	0
By students	3	1	1	1	0

4. SUBSCRIBING AND CO-OPERATING INSTITUTIONS

1942

Amherst College
Atlanta University
Beth Israel Hospital
Biological Institute, Philadelphia, Pennsylvania
Bowdoin College
Brooklyn College
Brown University
Bryn Mawr College
Canisius College
College of Physicians and Surgeons
Columbia University
Cornell University
Cornell University Medical College
Drew University
Duke University
Fordham University
Frick Educational Commission
Goucher College
Harvard University
Harvard University Medical School
Heidelburg College
Hunter College
Industrial and Engineering Chemistry, of the American Chemical Society
John and Mary Markle Foundation
Johns Hopkins University
Julius Rosenwald Fund
Eli Lilly and Co.
Long Island University
Marine Studios, Inc.
Massachusetts State College
Morehouse College
Mount Sinai Hospital, New York City
National Research Council
New York State Department of Health
New York University
New York University College of Medicine
New York University Washington Square College
Oberlin College
Ohio State University
Pennsylvania College for Women
Princeton University
Radcliffe College
Rockefeller Institute for Medical Research
Russell Sage College
Rutgers University
St. Joseph's Seminary, Dunwoodie, New York
Smith College
State University of Iowa
Sweet Briar College
Syracuse University
Tufts College
Union College
University of Cincinnati
University of Illinois
University of Maryland Medical School
University of Missouri
University of Pennsylvania
University of Pennsylvania School of Medicine
University of Pittsburgh
University of Rochester
Vanderbilt University
Vanderbilt University Medical School
Vassar College
Villanova College
Washington University
Wellesley College
Wesleyan University
Wheaton College
Woods Hole Oceanographic Institution
Yale University

5. EVENING LECTURES, 1942

Friday, June 26
Dr. Michael Heidelberger ...."Biological Aspects of Immunity and Complement Action."
Friday, July 3
Dr. Donald R. Griffin ...."Echo Sounding by Flying Bats."
Friday, July 10
Dr. R. Ruggles Gates ...."The Nucleolus and Phylogeny."
Friday, July 17
Dr. E. Newton Harvey ...."Animal Luminescence."
Friday, July 24
Mr. Per Höst ...."Norway Fights On."
Friday, July 31

Dr. David Nachmansohn	"On the Mechanism of Transmission of Nerve Impulses."
Friday, August 7	
Prof. Selman A. Waksman	"Science in Soviet Russia on the Eve of the World War."
Friday, August 14	
Dr. A. Glenn Richards, Jr.	"Electron Microscope Studies of Insect Structures and Tissues."
Friday, August 21	
Dr. Robert F. Griggs	"Timber Lines as Indices of Climatic Change."
Thursday, August 27	
Dr. Oscar W. Richards	"The Precision of Sectioning with a Microtome."
Friday, August 28	
Dr. C. W. Metz	"Evolutionary Chromosome Changes in Sciara as Shown by the Giant Salivary Gland Chromosomes."

6. SHORTER SCIENTIFIC PAPERS, 1942

Tuesday, July 21	
Dr. K. C. Fisher and Grace W. Scott	"The physiological basis of temperature 'selection' by fish."
Dr. J. R. Stern and K. C. Fisher	"The action of narcotics on oxygen consumption of resting and caffeinized frog muscle."
Dr. A. C. Giese and E. L. Tatum	"Effects of vitamins of the B-complex on respiration of Neurospora mutants."
Tuesday, August 4	
Mr. Sol Spiegelman	"Differential effects on the mass and time of appearance of regenerants in Tubularia."
Miss Florence Moog	"Some effects of temperature in the regeneration of Tubularia."
Dr. Mordecai Gabriel	"The effect of temperature on vertebral variations in Fundulus heteroclitus."
Tuesday, August 18	
Dr. Dorothy Wrinch	"The structure of biologically active membranes."
Dr. Douglas Marsland	"The contractile mechanism in unicellular melanophores."
Dr. E. H. Runyon	"The aggregation of separate cells of Dictyostelium to form a multicellular body."
Tuesday, August 25	
Dr. G. M. Everett	"Vitamin B_1 deficiency in the cat." Motion pictures in color.
Dr. T. H. Bissonnette	"Experimental modification of molts, and color-changes by controlled lighting of the Bonaparte weasel."

7. MEMBERS OF THE CORPORATION, 1942

1. Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Maryland.
Beckwith, Dr. Cora J., Vassar College, Poughkeepsie, New York.
Billings, Mr. R. C., 66 Franklin Street, Boston, Massachusetts.
Calvert, Dr. Philip P., University of Pennsylvania, Philadelphia, Pennsylvania.
Cole, Dr. Leon J., College of Agriculture, Madison, Wisconsin.
Conklin, Prof. Edwin G., Princeton University, Princeton, New Jersey.
Cowdry, Dr. E. V., Washington University, St. Louis, Missouri.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Massachusetts.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Gardiner, Mrs. E. G., Woods Hole, Massachusetts.
Jackson, Mr. Chas. C., 24 Congress Street, Boston, Massachusetts.
Jackson, Miss M. C., 88 Marlboro Street, Boston, Massachusetts.
King, Mr. Chas. A.
Kingsbury, Prof. B. F., Cornell University, Ithaca, New York.
Lewis, Prof. W. H., Johns Hopkins University, Baltimore, Maryland.
Lowell, Mr. A. L., 17 Quincy Street, Cambridge, Massachusetts.
Means, Dr. J. H., 15 Chestnut Street, Boston, Massachusetts.
Moore, Dr. George T., Missouri Botanical Gardens, St. Louis, Missouri.
Moore, Dr. J. Percy, University of Pennsylvania, Philadelphia, Pa.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Streets, New York City, New York.
Morgan, Mrs. T. H., Pasadena, California.
Morgan, Prof. T. H., Director of Biological Laboratory, California Institute of Technology, Pasadena, California.
Morrill, Dr. A. D., Hamilton College, Clinton, New York.
Noyes, Miss Eva J.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pennsylvania.
Scott, Dr. Ernest L., Columbia University, New York City, New York.
Sears, Dr. Henry F., 86 Beacon Street, Boston, Massachusetts.
Shedd, Mr. E. A.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City, New York.
Treadwell, Prof. A. L., Vassar College, Poughkeepsie, New York.
Trelease, Prof. William, University of Illinois, Urbana, Illinois.
Waite, Prof. F. C., 144 Locust Street, Dover, New Hampshire.
Wallace, Louise B., 359 Lytton Avenue, Palo Alto, California.

2. Regular Members

Abramowitz, Dr. Alexander A., Biological Laboratories, Harvard University, Cambridge, Massachusetts.
Adams, Dr. A. Elizabeth, Mount Holyoke College, South Hadley, Massachusetts.
Addison, Dr. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.

Adolph, Dr. Edward F., University of Rochester Medical School, Rochester, New York.

Albaum, Dr. Harry G., 3115 Avenue I, Brooklyn, New York.

Allee, Dr. W. C., The University of Chicago, Chicago, Illinois.

Amberson, Dr. William R., Department of Physiology, University of Maryland, School of Medicine, Lombard and Greene Streets, Baltimore, Maryland.

Anderson, Dr. Rubert S., Memorial Hospital, 444 East 68th Street, New York City, New York.

Angerer, Dr. Clifford A., Department of Physiology, Ohio State University, Columbus, Ohio.

Armstrong, Dr. Philip B., College of Medicine, Syracuse University, Syracuse, New York.

Austin, Dr. Mary L., Wellesley College, Wellesley, Massachusetts.

Baitsell, Dr. George A., Yale University, New Haven, Connecticut.

Baker, Dr. H. B., Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.

Ballard, Dr. William W., Dartmouth College, Hanover, New Hampshire.

Ballentine, Dr. Robert, Columbia University, Department of Zoology, New York City, New York.

Ball, Dr. Eric G., Department of Biological Chemistry, Harvard University Medical School, Boston, Massachusetts.

Bard, Prof. Philip, Johns Hopkins Medical School, Baltimore, Maryland.

Barron, Dr. E. S. Guzman, Department of Medicine, The University of Chicago, Chicago, Illinois.

Barth, Dr. L. G., Department of Zoology, Columbia University, New York City, New York.

Beadle, Dr. G. W., School of Biological Sciences, Stanford University, California.

Beams, Dr. Harold W., Department of Zoology, State University of Iowa, Iowa City, Iowa.

Behre, Dr. Elinor H., Louisiana State University, Baton Rouge, Louisiana.

Bigelow, Dr. H. B., Museum of Comparative Zoology, Cambridge, Massachusetts.

Bigelow, Prof. R. P., Massachusetts Institute of Technology, Cambridge, Massachusetts.

Binford, Prof. Raymond, Buck Creek Camp, Marion, North Carolina.

Bissonnette, Dr. T. Hume, Trinity College, Hartford, Connecticut.

Blanchard, Prof. Kenneth C., Washington Square College, New York University, New York City, New York.

Bodine, Dr. J. H., Department of Zoology, State University of Iowa, Iowa City, Iowa.

Boring, Dr. Alice M., Yenching University, Peking, China.

Bradley, Prof. Harold C., University of Wisconsin, Madison, Wisconsin.

Brodie, Mr. Donald M., 522 Fifth Avenue, New York City, New York.

Bronfenbrenner, Dr. Jacques J., Department of Bacteriology, Washington University Medical School, St. Louis, Missouri.

Brooks, Dr. Matilda M., University of California, Department of Zoology, Berkeley, California.

Brooks, Dr. S. C., University of California, Berkeley, California.

Brown, Dr. Dugald E. S., New York University, College of Dentistry, 209 East 23d Street, New York City, New York.
Brown, Dr. Frank A., Jr., Department of Zoology, Northwestern University, Evanston, Illinois.
Buckingham, Miss Edith N., Sudbury, Massachusetts.
Buck, Dr. John B., Department of Zoology, University of Rochester, Rochester, New York.
Budington, Prof. R. A., Winter Park, Florida.
Bullington, Dr. W. E., Randolph-Macon College, Ashland, Virginia.
Bumpus, Prof. H. C., Duxbury, Massachusetts.
Byrnes, Dr. Esther F., 1803 North Camac Street, Philadelphia, Pennsylvania.
Calkins, Prof. Gary N., Columbia University, New York City, New York.
Cannan, Prof. R. K., New York University College of Medicine, 477 First Avenue, New York City, New York.
Carlson, Prof. A. J., Department of Physiology, The University of Chicago, Chicago, Illinois.
Carothers, Dr. E. Eleanor, 134 Avenue C. East, Kingman, Kansas.
Carpenter, Dr. Russell L., Tufts College, Tufts College, Massachusetts.
Carroll, Prof. Mitchell, Franklin and Marshall College, Lancaster, Pennsylvania.
Carver, Prof. Gail L., Mercer University, Macon, Georgia.
Cattell, Dr. McKeen, Cornell University Medical College, 1300 York Avenue, New York City, New York.
Cattell, Prof. J. McKeen, Garrison-on-Hudson, New York.
Cattell, Mr. Ware, Smithsonian Institution Building, Washington, D. C.
Chambers, Dr. Robert, Washington Square College, New York University, Washington Square, New York City, New York.
Chase, Dr. Aurin M., Princeton University, Princeton, New Jersey.
Cheney, Dr. Ralph H., Biology Department, Long Island University, Brooklyn, New York.
Chidester, Prof. F. E., Auburndale, Massachusetts.
Child, Prof. C. M., Jordan Hall, Stanford University, California.
Churney, Dr. Leon, 155 Powell Lane, Upper Darby, Pennsylvania.
Claff, Mr. C. Lloyd, Department of Biology, Brown University, Providence, Rhode Island.
Clark, Prof. E. R., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.
Clark, Dr. Leonard B., Department of Biology, Union College, Schenectady, New York.
Cleland, Prof. Ralph E., Indiana University, Bloomington, Indiana.
Clowes, Dr. G. H. A., Eli Lilly and Company, Indianapolis, Indiana.
Coe, Prof. W. R., Yale University, New Haven, Connecticut.
Cohn, Dr. Edwin J., 183 Brattle Street, Cambridge, Massachusetts.
Cole, Dr. Elbert C., Department of Biology, Williams College, Williamstown, Massachusetts.
Cole, Dr. Kenneth S., College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York City, New York.
Collett, Dr. Mary E., Western Reserve University, Cleveland, Ohio.

Colton, Prof. N. S., Box 601, Flagstaff, Arizona.
Cooper, Dr. Kenneth W., Department of Biology, Princeton University, Princeton, New Jersey.
Copeland, Prof. Manton, Bowdoin College, Brunswick, Maine.
Costello, Dr. Donald P., Department of Zoology, University of North Carolina, Chapel Hill, North Carolina.
Costello, Dr. Helen Miller, Department of Zoology, University of North Carolina, Chapel Hill, North Carolina.
Crampton, Prof. H. E., Barnard College, Columbia University, New York City, New York.
Crowell, Dr. P. S., Jr., Department of Zoology, Miami University, Oxford, Ohio.
Curtis, Dr. Maynie R., 377 Dexter Trail, Mason, Michigan.
Curtis, Prof. W. C., University of Missouri, Columbia, Missouri.
Dan, Dr. Katsuma, Misaki Biological Station, Misaki, Japan.
Davis, Dr. Donald W., College of William and Mary, Williamsburg, Virginia.
Dawson, Dr. A. B., Harvard University, Cambridge, Massachusetts.
Dawson, Dr. J. A., The College of the City of New York, New York City, New York.
Dederer, Dr. Pauline H., Connecticut College, New London, Connecticut.
Demerec, Dr. M., Carnegie Institution of Washington, Cold Spring Harbor, Long Island, New York.
Diller, Dr. William F., 1016 South 45th Street, Philadelphia, Pennsylvania.
Dodds, Prof. G. S., Medical School, University of West Virginia, Morgantown, West Virginia.
Dolley, Prof. William L., University of Buffalo, Buffalo, New York.
Donaldson, Dr. John C., University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania.
DuBois, Dr. Eugene F., Cornell University Medical College, 1300 York Avenue, New York City, New York.
Duggar, Dr. Benjamin M., University of Wisconsin, Madison, Wisconsin.
Dungay, Dr. Neil S., Carleton College, Northfield, Minnesota.
Duryee, Dr. William R., Department of Biology, Washington Square College, New York University, New York City, New York.
Edwards, Dr. D. J., Cornell University Medical College, 1300 York Avenue, New York City, New York.
Ellis, Dr. F. W., Monson Massachusetts.
Evans, Dr. Titus C., 723 Kirkwood, Iowa City, Iowa.
Failla, Dr. G., College of Physicians and Surgeons, 630 West 168th Street, New York City, New York.
Fauré-Fremiet, Prof. Emmanuel, Collège de France, Paris, France.
Ferguson, Dr. James K. W., Department of Pharmacology, University of Toronto, Ontario, Canada.
Figge, Dr. F. H. J., 4636 Schenley Road, Baltimore, Maryland.
Fischer, Dr. Ernst, Department of Physiology, Medical College of Virginia, Richmond, Virginia.
Fisher, Dr. Jeanne M., Department of Biochemistry, University of Toronto, Toronto, Canada.

Fisher, Dr. Kenneth C., Department of Biology, University of Toronto, Toronto, Canada.

Fleisher, Dr. Moyer S., 20 North Kingshighway, St. Louis, Missouri.

Forbes, Dr. Alexander, Harvard University Medical School, Boston, Massachusetts.

Frisch, Dr. John A., Canisius College, Buffalo, New York.

Furth, Dr. Jacob, Cornell University Medical College, 1300 York Avenue, New York City, New York.

Gage, Prof. S. H., Cornell University, Ithaca, New York.

Galtsoff, Dr. Paul S., 420 Cumberland Avenue, Somerset, Chevy Chase, Maryland.

Garrey, Prof. W. E., Vanderbilt University Medical School, Nashville, Tennessee.

Geiser, Dr. S. W., Southern Methodist University, Dallas, Texas.

Gerard, Prof. R. W., The University of Chicago, Chicago, Illinois.

Glaser, Prof. O. C., Amherst College, Amherst, Massachusetts.

Goldforb, Prof. A. J., College of the City of New York, Convent Avenue and 139th Street, New York City, New York.

Goodrich, Prof. H. B., Wesleyan University, Middletown, Connecticut.

Gottschall, Dr. Gertrude Y., 1630 Rhode Island Avenue, N.W., Washington, D. C.

Graham, Dr. J. Y., University of Alabama, University, Alabama.

Grand, Constantine G., Biology Department, Washington Square College, New York University, Washington Square, New York City, New York.

Grave, Prof. B. H., DePauw University, Greencastle, Indiana.

Grave, Prof. Caswell, Washington University, St. Louis, Missouri.

Gray, Prof. Irving E., Duke University, Durham, North Carolina.

Gregory, Dr. Louise H., Barnard College, Columbia University, New York City, New York.

Gudernatsch, J. Fredrick, New York University, 100 Washington Square, New York City, New York.

Guthrie, Dr. Mary J., University of Missouri, Columbia, Missouri.

Guyer, Prof. M. F., University of Wisconsin, Madison, Wisconsin.

Hague, Dr. Florence, Sweet Briar College, Sweet Briar, Virginia.

Hall, Prof. Frank G., Duke University, Durham, North Carolina.

Hamburger, Dr. Viktor, Department of Zoology, Washington University, St. Louis, Missouri.

Hance, Dr. Robert T., Department of Biology, Duquesne University, Pittsburgh, Pennsylvania.

Hargitt, Prof. George T., Department of Zoology, Duke University, Durham, North Carolina.

Harman, Dr. Mary T., Kansas State Agricultural College, Manhattan, Kansas.

Harnly, Dr. Morris H., Washington Square College, New York University, New York City, New York.

Harper, Prof. R. A., R. No. 5, Bedford, Virginia.

Harrison, Prof. Ross G., Yale University, New Haven, Connecticut.

Hartline, Dr. H. Keffer, University of Pennsylvania, Philadelphia, Pennsylvania.

Hartman, Dr. Frank A., Hamilton Hall, Ohio State University, Columbus, Ohio.

Harvey, Dr. E. Newton, Guyot Hall, Princeton University, Princeton, New Jersey.

Harvey, Dr. Ethel Browne, 48 Cleveland Lane, Princeton, New Jersey.

Hayden, Dr. Margaret A., Wellesley College, Wellesley, Massachusetts.

Hayes, Dr. Frederick R., Zoological Laboratory, Dalhousie University, Halifax, Nova Scotia.

Haywood, Dr. Charlotte, Mount Holyoke College, South Hadley, Massachusetts.

Hazen, Dr. T. E., Barnard College, Columbia University, New York City, New York.

Hecht, Dr. Selig, Columbia University, New York City, New York.

Heilbrunn, Dr. L. V., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.

Hendee, Dr. Esther Crissey, Russell Sage College, Troy, New York.

Henshaw, Dr. Paul S., National Cancer Institute, Bethesda, Maryland.

Hess, Prof. Walter N., Hamilton College, Clinton, New York.

Hibbard, Dr. Hope, Department of Zoology, Oberlin College, Oberlin, Ohio.

Hill, Dr. Samuel E., Department of Biology, Russell Sage College, Troy, New York.

Hinrichs, Dr. Marie, Department of Physiology and Health Education, South Illinois Normal University, Carbondale, Illinois.

Hisaw, Dr. F. L., Harvard University, Cambridge, Massachusetts.

Hoadley, Dr. Leigh, Harvard University, Cambridge, Massachusetts.

Höber, Dr. Rudolf, University of Pennsylvania, Philadelphia, Pennsylvania.

Hodge, Dr. Charles, IV, Temple University, Department of Zoology, Philadelphia, Pennsylvania.

Hogue, Dr. Mary J., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.

Hollaender, Dr. Alexander, c/o National Institute of Health, Laboratory of Industrial Hygiene, Bethesda, Maryland.

Hooker, Prof. Davenport, University of Pittsburgh, School of Medicine, Department of Anatomy, Pittsburgh, Pennsylvania.

Hopkins, Dr. Dwight L., Mundelein College, 6363 Sheridan Road, Chicago, Illinois.

Hopkins, Dr. Hoyt S., New York University, College of Dentistry, New York City, New York.

Howe, Dr. H. E., 1155 16th St., N.W., American Chemical Society Bldg., Washington, D. C.

Howland, Dr. Ruth B., Washington Square College, New York University, Washington Square East, New York City, New York.

Hoyt, Dr. William D., Washington and Lee University, Lexington, Virginia.

Hyman, Dr. Libbie H., American Museum of Natural History, New York City, New York.

Irving, Prof. Laurence, Swarthmore College, Swarthmore, Pennsylvania.

Iselin, Mr. Columbus O'D., Woods Hole, Massachusetts.

Jacobs, Prof. Merkel H., School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

Jenkins, Dr. George B., 30 Gallatin Street, N.W., Washington, D. C.

Jennings, Prof. H. S., Department of Zoology, University of California, Los Angeles, California.
Jewett, Prof. J. R., 44 Francis Avenue, Cambridge, Massachusetts.
Johlin, Dr. J. M., Vanderbilt University Medical School, Nashville, Tennessee.
Jones, Dr. E. Ruffin, Jr., College of William and Mary, Williamsburg, Virginia.
Kaufmann, Prof. B. P., Carnegie Institution, Cold Spring Harbor, Long Island, New York.
Kempton, Prof. Rudolf T., Vassar College, Poughkeepsie, New York.
Kidder, Dr. George W., Brown University, Providence, Rhode Island.
Kille, Dr. Frank R., Swarthmore College, Swarthmore, Pennsylvania.
Kindred, Dr. J. E., University of Virginia, Charlottesville, Virginia.
King, Dr. Helen D., Wistar Institute of Anatomy and Biology, 36th Street and Woodland Avenue, Philadelphia, Pennsylvania.
King, Dr. Robert L., State University of Iowa, Iowa City, Iowa.
Knowlton, Prof. F. P., Syracuse University, Syracuse, New York.
Kopac, Dr. M. J., Washington Square College, New York University, New York City, New York.
Korr, Dr. I. M., Department of Physiology, New York University, College of Medicine, 477 First Avenue, New York City, New York.
Krahl, Dr. M. E., Lilly Research Laboratories, Indianapolis, Indiana.
Krieg, Dr. Wendell J. S., New York University, College of Medicine, 477 First Avenue, New York City, New York.
Lancefield, Dr. D. E., Queens College, Flushing, New York.
Lancefield, Dr. Rebecca C., Rockefeller Institute, 66th Street and York Avenue, New York City, New York.
Lange, Dr. Mathilde M., Wheaton College, Norton, Massachusetts.
Lewis, Prof. I. F., University of Virginia, Charlottesville, Virginia.
Lillie, Prof. Frank R., The University of Chicago, Chicago, Illinois.
Lillie, Prof. Ralph S., The University of Chicago, Chicago, Illinois.
Loeb, Prof. Leo, 40 Crestwood Drive, St. Louis, Missouri.
Loewi, Prof. Otto, 155 East 93d Street, New York City, New York.
Lowther, Mrs. Florence deL., Barnard College, Columbia University, New York City, New York.
Lucas, Dr. Alfred M., Zoological Laboratory, Iowa State College, Ames, Iowa.
Lucas, Dr. Miriam Scott, Department of Zoology, Iowa State College, Ames, Iowa.
Lucké, Prof. Balduin, University of Pennsylvania, Philadelphia, Pennsylvania.
Lynch, Dr. Clara J., Rockefeller Institute, 66th Street and York Avenue, New York City, New York.
Lynch, Dr. Ruth Stocking, Maryland State Teachers College, Towson, Maryland.
Lynn, Dr. William G., Department of Biology, The Catholic University of America, Washington, D. C.
MacDougall, Dr. Mary S., Agnes Scott College, Decatur, Georgia.
MacLennan, Dr. Ronald F., 174 Forest Street, Oberlin, Ohio.
MacNaught, Mr. Frank M., Marine Biological Laboratory, Woods Hole, Massachusetts.

McClung, Prof. C. E., 417 Harvard Avenue, Swarthmore, Pennsylvania.
McCouch, Dr. Margaret Sumwalt, University of Pennsylvania Medical School, Philadelphia, Pa.
McGregor, Dr. J. H., Columbia University, New York City, New York.
Macklin, Dr. Charles C., School of Medicine, University of Western Ontario, London, Canada.
Magruder, Dr. Samuel R., Department of Anatomy, Tufts Medical School, Boston, Massachusetts.
Malone, Prof. E. F., College of Medicine, University of Cincinnati, Department of Anatomy, Cincinnati, Ohio.
Manwell, Dr. Reginald D., Syracuse University, Syracuse, New York.
Marsland, Dr. Douglas A., Washington Square College, New York University, New York City, New York.
Martin, Prof. E. A., Department of Biology, Brooklyn College, Bedford Avenue and Avenue H, Brooklyn, New York.
Mast, Prof. S. O., Johns Hopkins University, Baltimore, Maryland.
Mathews, Prof. A. P., University of Cincinnati, Cincinnati, Ohio.
Matthews, Dr. Samuel A., Thompson Biological Laboratory, Williams College, Williamstown, Massachusetts.
Mavor, Prof. James W., Union College, Schenectady, New York.
Mazia, Dr. Daniel, Department of Zoology, University of Missouri, Columbia, Missouri.
Medes, Dr. Grace, Lankenau Research Institute, Philadelphia, Pennsylvania.
Meigs, Mrs. E. B., 1736 M Street, N.W., Washington, D. C.
Menkin, Dr. Valy, Harvard Medical School, Boston, Massachusetts.
Metz, Prof. Charles W., University of Pennsylvania, Philadelphia, Pennsylvania.
Michaelis, Dr. Leonor, Rockefeller Institute, 66th Street and York Avenue, New York City, New York.
Miller, Dr. J. A., Division of Anatomy, College of Medicine, University of Tennessee, Memphis, Tennessee.
Minnich, Prof. D. F., Department of Zoology, University of Minnesota, Minneapolis, Minnesota.
Mitchell, Dr. Philip H., Brown University, Providence, Rhode Island.
Moore, Dr. Carl R., The University of Chicago, Chicago, Illinois.
Morgan, Dr. Isabel M., Rockefeller Institute, York Avenue at 66th Street, New York City, New York.
Morgulis, Dr. Sergius, University of Nebraska, Omaha, Nebraska.
Morrill, Prof. C. V., Cornell University Medical College, 1300 York Avenue, New York City, New York.
Moser, Dr. Floyd, Department of Biology, University of Alabama, University, Alabama.
Muller, Prof. H. J., Amherst College, Amherst, Massachusetts.
Navez, Dr. Albert E., Department of Biology, Milton Academy, Milton, Massachusetts.
Newman, Prof. H. H., 173 Devon Drive, Clearwater, Florida.
Nichols, Dr. M. Louise, Rosemont, Pennsylvania.
Nonidez, Dr. José F., Cornell University Medical College, 1300 York Avenue, New York City, New York.

Northrop, Dr. John H., The Rockefeller Institute, Princeton, New Jersey.

Okkelberg, Dr. Peter, Department of Zoology, University of Michigan, Ann Arbor, Michigan.

Oppenheimer, Dr. Jane M., Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania.

Osburn, Prof. R. C., Ohio State University, Columbus, Ohio.

Osterhout, Prof. W. J. V., Rockefeller Institute, 66th Street and York Avenue, New York City, New York.

Osterhout, Mrs. Marian Irwin, Rockefeller Institute, 66th Street and York Avenue, New York City, New York.

Packard, Dr. Charles, Marine Biological Laboratory, Woods Hole, Massachusetts.

Page, Dr. Irvine H., Lilly Laboratory Clinical Research, Indianapolis City Hospital, Indianapolis, Indiana.

Pappenheimer, Dr. A. M., Columbia University, New York City, New York.

Parker, Prof. G. H., Harvard University, Cambridge, Massachusetts.

Parmenter, Dr. C. L., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.

Parpart, Dr. Arthur K., Princeton University, Princeton, New Jersey.

Patten, Dr. Bradley M., University of Michigan Medical School, Ann Arbor, Michigan.

Payne, Prof. F., University of Indiana, Bloomington, Indiana.

Peebles, Prof. Florence, Lewis and Clark College, Portland, Oregon.

Pinney, Dr. Mary E., Milwaukee-Downer College, Milwaukee, Wisconsin.

Plough, Prof. Harold H., Amherst College, Amherst, Massachusetts.

Pollister, Dr. A. W., Columbia University, New York City, New York.

Pond, Dr. Samuel E., 1203 Enfield Street, Thompsonville, Connecticut.

Pratt, Dr. Frederick H., Boston University, School of Medicine, Boston, Massachusetts.

Prosser, Dr. C. Ladd, University of Illinois, Urbana, Illinois.

Rand, Dr. Herbert W., Harvard University, Cambridge, Massachusetts.

Rankin, Dr. John S., Zoology Department, University of Washington, Seattle, Washington.

Redfield, Dr. Alfred C., Harvard University, Cambridge, Massachusetts.

Renshaw, Prof. Birdsey, 4600 Harling Lane, Bethesda, Maryland.

deRenyi, Dr. George S., Department of Anatomy, University of Pennsylvania, Philadelphia, Pennsylvania.

Reznikoff, Dr. Paul, Cornell University Medical College, 1300 York Avenue, New York City, New York.

Rice, Prof. Edward L., Ohio Wesleyan University, Delaware, Ohio.

Richards, Prof. A., University of Oklahoma, Norman, Oklahoma.

Richards, Prof. A. G., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.

Richards, Dr. O. W., Research Department, Spencer Lens Company, 19 Doat Street, Buffalo, New York.

Riggs, Lawrason, Jr., 120 Broadway, New York City, New York.

Rogers, Prof. Charles G., Oberlin College, Oberlin, Ohio.

Romer, Dr. Alfred S., Harvard University, Cambridge, Massachusetts.

Root, Dr. R. W., Department of Biology, College of the City of New York, Convent Avenue and 139th Street, New York City, New York.

Root, Dr. W. S., College of Physicians and Surgeons, Department of Physiology, 630 West 168th Street, New York City, New York.

Ruebush, Dr. T. K., Naval Medical School, National Naval Medical Center, Bethesda, Maryland.

Rugh, Dr. Roberts, Department of Biology, Washington Square College, New York University, New York City, New York.

Saslow, Dr. George, 72 Grozier Road, Cambridge, Massachusetts.

Sayles, Dr. Leonard P., Department of Biology, College of the City of New York, 139th Street and Convent Avenue, New York City, New York.

Schaeffer, Dr. Asa A., Biology Department, Temple University, Philadelphia, Pennsylvania.

Schechter, Dr. Victor, College of the City of New York, 139th Street and Convent Avenue, New York City, New York.

Schmidt, Dr. L. H., Christ Hospital, Cincinnati, Ohio.

Schmitt, Prof. F. O., Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Schotté, Dr. Oscar E., Department of Biology, Amherst College, Amherst, Massachusetts.

Schrader, Dr. Franz, Department of Zoology, Columbia University, New York City, New York.

Schrader, Dr. Sally Hughes, Department of Zoology, Columbia University, New York City, New York.

Schramm, Prof. J. R., University of Pennsylvania, Philadelphia, Pennsylvania.

Scott, Dr. Allan C., Union College, Schenectady, New York.

Scott, Prof. William B., 7 Cleveland Lane, Princeton, New Jersey.

Scott, Sister Florence Marie, Professor of Biology, Seton Hill College, Greensburg, Pennsylvania.

Semple, Mrs. R. Bowling, 140 Columbia Heights, Brooklyn, New York.

Severinghaus, Dr. Aura E., Department of Anatomy, College of Physicians and Surgeons, 630 West 168th Street, New York City, New York.

Shapiro, Dr. Herbert, Radiation Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Shelford, Prof. V. E., Vivarium, Wright and Healey Streets, Champaign, Illinois.

Shull, Prof. A. Franklin, University of Michigan, Ann Arbor, Michigan.

Shumway, Dr. Waldo, University of Illinois, Urbana, Illinois.

Sichel, Dr. Ferdinand J. M., University of Vermont, Burlington, Vermont.

Sichel, Mrs. F. J. M., 35 Henderson Terrace, Burlington, Vermont.

Sinnott, Dr. E. W., Osborn Botanical Laboratory, Yale University, New Haven, Connecticut.

Slifer, Dr. Eleanor H., Department of Zoology, State University of Iowa, Iowa City, Iowa.

Smith, Dr. Dietrich Conrad, Department of Physiology, University of Maryland School of Medicine, Lombard and Greene Streets, Baltimore, Maryland.

Snyder, Prof. L. H., Ohio State University, Department of Zoology, Columbus, Ohio.

Sollman, Dr. Torald, Western Reserve University, Cleveland, Ohio.

Sonneborn, Dr. T. M., Department of Zoology, Indiana University, Bloomington, Indiana.

Speidel, Dr. Carl C., University of Virginia, University, Virginia.

Stabler, Dr. Robert M., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.

Stark, Dr. Mary B., 1 East 105th Street, New York City, New York.

Steinbach, Dr. H. Burr, Department of Zoology, Washington University, St. Louis, Missouri.

Stern, Dr. Curt, Department of Zoology, University of Rochester, Rochester, New York.

Stern, Dr. Kurt G., Overly Biochemical Research Foundation, 254 W. 31st Street, New York City, New York.

Stewart, Dr. Dorothy R., Skidmore College, Saratoga Springs, New York.

Stokey, Dr. Alma G., Department of Botany, Mount Holyoke College, South Hadley, Massachusetts.

Strong, Prof. O. S., College of Physicians and Surgeons, Columbia University, New York City, New York.

Stunkard, Dr. Horace W., New York University, University Heights, New York.

Sturtevant, Dr. Alfred H., California Institute of Technology, Pasadena, California.

Summers, Dr. Francis Marion, Department of Biology, College of the City of New York, New York City, New York.

Swett, Dr. Francis H., Duke University Medical School, Durham, North Carolina.

Taft, Dr. Charles H., Jr., University of Texas Medical School, Galveston, Texas.

Tashiro, Dr. Shiro, Medical College, University of Cincinnati, Cincinnati, Ohio.

Taylor, Dr. C. V., Leland Stanford University, Leland Stanford, California.

Taylor, Dr. William R., University of Michigan, Ann Arbor, Michigan.

TeWinkel, Dr. L. E., Department of Zoology, Smith College, Northampton, Massachusetts.

Turner, Dr. Abby H., Department of Physiology, Mount Holyoke College, South Hadley, Massachusetts.

Turner, Prof. C. L., Northwestern University, Evanston, Illinois.

Tyler, Dr. Albert, California Institute of Technology, Pasadena, California.

Uhlenhuth, Dr. Eduard, University of Maryland, School of Medicine, Baltimore, Maryland.

Unger, Dr. W. Byers, Dartmouth College, Hanover, New Hampshire.

Visscher, Dr. J. Paul, Western Reserve University, Cleveland, Ohio.

Wald, Dr. George, Biological Laboratories, Harvard University, Cambridge, Massachusetts.

Ward, Prof. Henry B., 1201 W. Nevada, Urbana, Illinois..

Warren, Dr. Herbert S., 1405 Greywall Lane, Overbrook Hills, Pennsylvania.

Waterman, Dr. Allyn J., Department of Biology, Williams College, Williamstown, Massachusetts.

Weiss, Dr. Paul A., Department of Zoology, The University of Chicago, Chicago, Illinois.

Wenrich, Dr. D. H., University of Pennsylvania, Philadelphia, Pennsylvania.

Whedon, Dr. A. D., North Dakota Agricultural College, Fargo, North Dakota.
Whitaker, Dr. Douglas M., P. O. Box 2514, Stanford University, California.
White, Dr. E. Grace, Wilson College, Chambersburg, Pennsylvania.
Whiting, Dr. Phineas W., Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.
Whitney, Dr. David D., University of Nebraska, Lincoln, Nebraska.
Wichterman, Dr. Ralph, Biology Department, Temple University, Philadelphia, Pennsylvania.
Wieman, Prof. H. L., University of Cincinnati, Cincinnati, Ohio.
Willier, Dr. B. H., Department of Biology, Johns Hopkins University, Baltimore, Maryland.
Wilson, Dr. J. W., Brown University, Providence, Rhode Island.
Witschi, Prof. Emil, Department of Zoology, State University of Iowa, Iowa City, Iowa.
Wolf, Dr. Ernst, Biological Laboratories, Harvard University, Cambridge, Massachusetts.
Woodruff, Prof. L. L., Yale University, New Haven, Connecticut.
Woodward, Dr. Alvalyn E., Zoology Department, University of Michigan, Ann Arbor, Michigan.
Yntema, Dr. C. L., Department of Anatomy, Cornell University Medical College, 1300 York Avenue, New York City, New York.
Young, Dr. B. P., Cornell University, Ithaca, New York.
Young, Dr. D. B., 7128 Hampden Lane, Bethesda, Maryland.

SEXUAL ISOLATION, MATING TYPES, AND SEXUAL RESPONSES TO DIVERSE CONDITIONS IN VARIETY 4, PARAMECIUM AURELIA [1]

T. M. SONNEBORN [2] AND RUTH V. DIPPELL

(*Department of Zoology, Indiana University, Bloomington*)

In previous publications (Sonneborn, 1938; 1939; 1943) the species *Paramecium aurelia* has been shown to consist of a number of sexually isolated and physiologically distinct groups of races. Their sexual isolation is perhaps sufficient ground for assigning these groups to different species; but as all are morphologically similar and conform to the description of the species *Paramecium aurelia*, it seems more practical for the present at least to designate them as varieties of this species. Each of these varieties consists of two classes of individuals that are morphologically identical but physiologically different. These two classes of individuals mate with each other, but neither class mates with other individuals of the same class or with either of the two classes that occur in any other variety of the species. The two classes of individuals within each variety are known as mating types and, in *P. aurelia*, they are designated by Roman numerals. The diverse varieties are designated by Arabic numerals.

The present paper is the first of a series dealing with the general biology and genetics of variety 4, containing the mating types VII and VIII. Each variety thus far studied has proven to be specially favorable for the study of certain problems of protozoan biology and genetics not so readily investigated in other varieties. As will appear in the course of this series of papers, investigations on variety 4 have yielded information on a number of important problems. In this first paper of the series we set forth the foundation on which the work of the later papers is based: demonstration of the existence of variety 4, and an account of its mating types and the conditions under which they mate.

MATERIAL

Among the 53 races of *P. aurelia* collected from different sources in nature and studied in this laboratory, only the following four belong to variety 4:

Race 29 collected by Dr. R. F. Kimball from Ben's Run, Hebbville, Maryland, in 1938.

Race 32 collected by Dr. Kimball from a pond in Towson, Maryland, in 1938.

Race 47 collected by Dr. A. C. Giese from a pool across the Bay from Berkeley, California, and sent to me in February 1939.

Race 51 collected by Mrs. Aner Laubscher at Spencer, Indiana, in August 1939.

Before intensive study of these races began in the spring of 1942, they were maintained in quart jars of hay infusion to which boiled hay strips were added every month or two. In the course of this period, race 47 either changed one

---

[1] Contribution No. 318 from the Department of Zoology, Indiana University.

[2] Aided by a grant from the Rockefeller Foundation.

of its characters or was mislabelled, for in 1939 it produced a unique type of lethal action on other races and no trace of this action has appeared in our recent work. In the following studies, these four races were cultivated in desiccated lettuce infusion to which a pure culture of the bacterium *Aerobacter aerogenes* was added.

Occurrence, Sexual Isolation and Mating Types of Variety 4

In order to discover whether a race or group of races constitutes a new variety (in the sense in which this term is employed here, i.e., a sexually isolated group of races), it is required to demonstrate that it contains mating types which interbreed with each other but not with those in any other known variety. This is made possible by the fact that all the mating types so far found in *P. aurelia* ordinarily reproduce true to type during vegetative reproduction and so yield from a single individual a clone containing one mating type only. Samples of clones of unidentified races may then be mixed with samples of sexually reactive clones of each of the known mating types. If no mating occurs in any of these mixtures, this is evidence that the new races do not contain any of the known mating types; but the evidence is not convincing unless it is certain that the clones of the new races, as well as those of the known mating types, are in sexually reactive condition at the time the tests are carried out. This can be achieved only when the clones of the new races mate with each other in appropriate combinations.

Such an analysis was carried out on the four races discusssed in this paper. Clones of each of these races were mixed with sexually reactive cultures of each of the six known mating types (I, II, III, IV, V, and VI) and no mating resulted. As repeated trials gave the same result, the six known mating types seemed not to occur among the four new races. However, at that time mating also failed to occur in mixtures of different clones and different races of the four new races. Under such conditions, conclusive proof that they constituted a new variety could not be given; they might simply have been immature. In April 1942 this difficulty disappeared when mating was observed for the first time in race 32. As some of the individuals were coming together in preparation for conjugation, they were separated before they had time to unite firmly and cultures were grown from the isolated members of the split pairs. The resulting clones proved to be of unlike mating types for no mating occurred within either clone alone, but the characteristic clumping reaction and conjugation took place when samples of the two clones from a split pair were mixed together. The same clones, while in this reactive condition, failed to clump or conjugate with any of the six previously known mating types, although all of these were at the time in highly reactive sexual condition. Hence, there occur in race 32 two mating types unlike any of those previously known. They were therefore called mating types VII and VIII. All clones of race 32 available at that time, and subsequently, have been found to belong to either one or the other of these two mating types. When these two types were mixed with samples of clones of the remaining three races (29, 47 and 51), clumping and conjugation occurred in the mixtures with type VIII, but not in the mixtures with type VII. These three races therefore contained type VII only and all clones examined at that time in these three races were found to be of type VII. The four races 29, 32, 47 and 51 thus constitute a fourth variety with two new mating types VII and VIII.

Subsequently, and at a definitely known time, type VIII arose independently in race 51, but it has still not been found in races 29 or 47 in spite of a prolonged and intensive search for it. However, type VIII might well arise eventually in these races also as it has already done in the other two races.

Sexual Responses to Diverse Conditions in Variety 4

The nutritive conditions for conjugation appear to be the same in variety 4 as in the three previously described varieties: the animals must be neither very well fed nor completely starved, but in a declining nutritive condition. The strongest mating reactions take place when there are in progress, in the cultures to be mixed, the last fissions before the food supply is exhausted.

As diurnal periodicities in the occurrence of the mating reaction exist in two of the three previously described varieties of *P. aurelia* (Sonneborn, 1938; 1939), the possibility of its occurrence was examined in variety 4. For this purpose, cultures of the races 29 and 47 and cultures of each mating type in the races 32 and 51 were prepared by growing them for 6 days exposed to the light of a north window during the daylight hours. The plan was to mix samples of each of the type VIII cultures (from races 32 and 51) with each of the type VII cultures (from all four of the races) at four-hour intervals through at least one complete cycle of 24 hours. In order to be sure to have cultures in the proper nutritive condition at all times, the six original cultures were subcultured in triplicate the evening before the tests were to be made and the three subcultures of each original were fed in the ratio of 1 : 2 : 4 volumes of culture fluid. During the daylight hours there was no difficulty in making the required mixtures, but at night precautions had to be taken to avoid exposing the cultures to light in so far as possible. This was accomplished as follows. Samples of all the cultures to be mixed at night were put into depression slides before dark. The two depressions of each slide contained two cultures that were later to be mixed. There was a separate slide for each combination and each time of mixture, with ample duplicates for emergencies. All of these slides were placed in moist chambers and were covered at night with black cloth. At the time for mixture, a very dim flashlight was directed away from the culture dishes, the appropriate slides were removed from the moist chambers, and the fluid from one depression on each slide was pipetted into the other depression of the same slide. Two or three minutes later the mixture was examined under the microscope with the faint light from the flashlight. The mixtures were then returned to the cloth-covered moist chambers.

A complete set of eight mixtures was made every four hours beginning at 5:15 P.M. on February 13 and continuing until 9:15 P.M. on February 14 Additional sets were made on other days at various times from 8 A.M. to 10 P.M. The agglutinative mating reaction occurred at once in mixtures made at every one of the different hours tested. There was thus no indication of any diurnal periodicity in the mating reaction. In this respect variety 4 is like variety 1 and unlike varieties 2 and 3 (Sonneborn, 1938; 1939).

The relation of temperature to the occurrence of conjugation was studied in five series of experiments. In each series, the same eight combinations of cultures were brought together as in the preceding experiments on diurnal periodicity. In series 1, each of the six cultures was grown for 6 days at 9°, 16.5°, 20° and 25° C.; then a set of eight mixtures was made and retained at each temperature and duplicate sets from 9° and 16.5° were immediately placed at 25°. In series 2, the same cultures were grown for one day at 9°, 15.5°, 21° and 25.5°; mixtures were made as in series 1, duplicate sets of mixtures from the two lower temperatures again being placed at once at the highest temperature. In series 3, the same six cultures were grown for 13 days at 9°, 15.5° and 26°; then mixtures were made and retained at the same temperatures and duplicate sets of mixtures from the two lower temperatures were again placed at the highest temperature; in addition two extra sets of mixtures were made from the 26° cultures: one was immediately placed at 9° and the other at 15.5°. In series 4, cultures were grown for one day at 22°, 30° and 36°; one set of mixtures was made and retained at each temperature, one set from 30° and one from 36° was placed at 22° and two sets from 22° were placed at 30° and 36° respectively. In series 5 the six cultures were grown for several days at 21°, then five sets of mixtures were placed at 10°, 19°, 24.5°, 29° and 39°, respectively. We report first the results on mixtures retained at the temperatures at which the cultures were grown, then the results of changing the temperature at the time the mixtures were made.

Cultures Grown and Tested at 9° C. Three sets of eight mixtures between types VII and VIII (series 1, 2, and 3) were grown and tested at 9°. In 20 of these mixtures no conjugation occurred at all; in the other four mixtures (all from series 1) less than 3 per cent of the animals conjugated. The mixtures of series 1 were observed $8\frac{1}{2}$ hours; series 2, 31 hours; and series 3, 23 days. Thus at 9° conjugation occurs in but a small proportion of mixtures and among only small proportions of the animals in these.

Cultures Grown and Tested at 15.5° to 16.5° C. Three sets (series 1, 2, and 3) of eight mixtures each were grown and tested at this temperature. The first two sets reacted poorly: half of the 16 mixtures gave no conjugation at all and the other half gave only 1 to 3 per cent conjugation. In the third set, one mixture gave 50 per cent conjugation and the other seven gave 15 to 25 per cent. Thus conjugation occurs in more of the cultures and may occur in a much higher proportion of the animals of a culture at this temperature than at 9°.

Cultures Grown and Tested at 20° to 22° The 24 mixtures (series 1, 2 and 4) grown and tested at this temperature all gave large proportions of conjugants—30 per cent to 90 per cent—and most of them gave immediate strong agglutinative reactions at the time of mixture. The latter did not occur at all at the lower temperatures.

Cultures Grown and Tested at 25° to 26°. Of the 24 mixtures made at this temperature, four proved unsuitable for study. The remaining 20 gave 40 per cent to 90 per cent conjugation and most gave strong immediate agglutinative mating reactions at the time of mixture.

Cultures Grown and Tested at 30°. The eight mixtures (series 4) at this temperature all gave immediate strong mating reactions and high percentages of conjugants.

Cultures Grown and Tested at 36°. The eight mixtures at this temperature (series 4) gave from 2 to 20 per cent conjugation.

At 39° cultures could not be grown, but the effects of this temperature, as set forth below, were studied in cultures grown at lower temperatures and placed at 39° immediately after mixture.

From the preceding, it appears that the optimal temperatures for conjugation in variety 4 extend from 20° to 30°; that the amount of conjugation obtained is approximately the same throughout this range of temperature; that the amount decreases both as temperature rises and falls away from this range; and that it occurs but rarely at 9°.

In the following paragraphs are presented the results of changing temperature at the time cultures of types VII and VIII are mixed together. The changes of temperature investigated were: (*a*) changes within the optimal range (20° to 30°); (*b*) changes from optimal to non-optimal temperatures; and (*c*) changes from non-optimal to optimal temperatures. The results, which are presented in this order, confirm and extend the conclusions in the preceding paragraph concerning the relation of temperature to the occurrence of conjugation in variety 4.

Changes of Temperature within the Optimal Range (20° to 30°). The following changes of temperature within the optimal range were investigated: cultures grown at 21°–22° were placed at the time of mixture at 24.5° (series 5), at 29° (series 5), and at 30° (series 4); and cultures grown at 30° were placed at the time of mixture at 22° (series 4). In each experiment, as in all of those that follow, a complete set of eight mixtures was again made in the way set forth in the preceding section. After all of these changes of temperature, the proportions of conjugants obtained in the mixtures were not significantly different from those obtained in other mixtures of the same cultures kept at the original temperatures. Hence, change of temperature within the optimal range has no effect on the proportion of conjugants obtained.

Changes from Optimal to Non-optimal Temperatures. When cultures of the two mating types were grown at a temperature within the range 20° to 30°, were mixed together and placed immediately at a temperature well outside this range, the proportions of animals that conjugated were always less than in corresponding controls retained after mixture at the original temperature.

In two experiments the temperature was raised from 21° or 22° to well over 30°. In one experiment, increase of temperature from 22° to 36° (series 4) resulted in no conjugation at all in two of the mixtures and in less than 12 per cent conjugation in the other six mixtures. The corresponding control mixtures retained at 22° gave in each of the eight mixtures from 30 to 90 per cent conjugation, or seven to eight times as much as in those placed at 36°. In the other experiment, increase of temperature from 21° to 39° resulted in no conjugation at all in any of the eight mixtures; but the corresponding eight control mixtures retained at 21° all conjugated in high proportions. Hence the upper limit of temperature for the occurrence of conjugation in variety 4 lies between 36° and 39°.

The temperature was lowered from 21° or 26° to well below 20° in three experiments. In one (series 5) the temperature was reduced from 21° to 10°. After 2 hours, the eight mixtures at 10° had less than half as many pairs of "conjugants" as the eight control mixtures retained at 21°. Moreover, while the pairs in the 21° mixtures were tightly united, those in the 10° mixtures were not. As will appear immediately, there is reason to believe that all of the latter pairs would have separated without having conjugated. Evidence for this was obtained in the second experiment (series 3) in which the temperature was reduced from 26° to 9°. Each of the eight control mixtures retained at 26° yielded more than 50 per cent of the animals tightly united in conjugation within 4 hours; but the eight mixtures at 9° contained at this time less than 10 per cent of the animals in pairs and these pairs were still loosely united. Soon thereafter all these pairs broke apart without having united in true conjugation and no other pairs formed, even loosely, within the next four days (compare with variety 1, Sonneborn, 1941). Reduction of temperature from over 20° to 10° or less thus suppresses conjugation just as does an increase of temperature to 39°. The third experiment (series 3) involved reduction of temperature from 26° to 15.5°. These eight mixtures each gave from 15 to 20 per cent conjugation, while each of the corresponding control mixtures at 26° gave more than 50 per cent conjugation within four hours.

All five of these experiments agree in showing that change from a temperature of 21° to 26° to one well below 20° or well above 30° results invariably in considerable reduction in the proportion of animals that conjugate. When the new temperature is as low as 10° or as high as 39°, conjugation is completely suppressed.

Changes from Non-optimal to Optimal Temperatures. Such changes include both reductions from very high to moderate temperatures and increases from very low to moderate temperatures. Both types of changes resulted in increases in the amount of conjugation. Thus, eight mixtures of cultures grown at 36° and placed immediately at 22° gave 10 to 70 per cent conjugation in 6½ hours, while corresponding control mixtures retained at 36° gave only 2 to 20 per cent conjugation in the same time. Further, three sets of cultures grown at 9° were mixed and placed at 25°–26°. All 24 of these mixtures yielded conjugants in proportions varying from 10 to 90 per cent; but 20 of the 24 control mixtures retained at 9° yielded no conjugants at all and the other four gave less than 3 per cent conjugation. Finally, three sets of cultures grown at 15.5°–16.5° were mixed and placed at 25°–26°. All 24 of these mixtures conjugated and gave higher proportions of conjugants than the corresponding controls kept at 15.5°–16.5°. For example, in one set, seven of the mixtures yielded 40 to 65 per cent conjugants while the corresponding controls yielded only 15 to 25 per cent; and the eighth mixture gave 75 per cent conjugation, its control only 50 per cent.

In general, when the temperature is changed at the time cultures of diverse mating type are mixed, the percentage of conjugation that results is unaffected if both the original and final temperatures are moderate (20° to 30°); it is greatly increased if the original temperature is extreme (36° and above, or 16° and below) and the final temperature moderate; and it is greatly decreased if the original temperature is moderate and the final temperature extreme. The optimal temperatures for the occurrence of conjugation in variety 4 are thus moderate (between 20° and 30°), regardless of whether mixtures are made from cultures grown at these or other temperatures. Conversely, as the temperature at which the mixtures are placed diverges from this optimum range (either above it or below), the percentage of conjugation decreases.

Discussion

The conditions for conjugation in variety 4 differ markedly from those for varieties 2 and 3 in the same ways that the conditions for conjugation in variety 1 do (Sonneborn, 1938; 1939). Both varieties 1 and 4 lack a diurnal periodicity in sexual reactivity. Both are able to conjugate over a wide range of temperatures. Both give smaller proportions of conjugants as temperature decreases below 20°. Both react to a sudden reduction of the temperature to 10° by discontinuing a mating reaction previously begun. Both are occasionally able to conjugate at this low temperature, if cultures of opposite types are grown at the same temperature some time before mixture. Nevertheless, varieties 1 and 4 do differ slightly in the conditions for conjugation; but the differences appear only at higher temperatures. Variety 4 gives maximum mating reactions between 20° and 30°, weak ones at 36° and fails to conjugate at 39°. Variety 1 gives maximum reactions between 20° and 38° and then suddenly fails to conjugate as the temperature rises to 40°. Thus, although conjugation occurs over practically the same range of temperature in the two varieties, the range of temperature for maximum sexual reactivity and the rate at which sexual reactivity decreases as the temperature rises above the optimum differ in the two varieties. At 36° the difference appears clearly: variety 1 gives a maximum reaction, while variety 4 conjugates but poorly. Thus it is possible to distinguish these four varieties of *P. aurelia* not only by their mating types, but also by the sexual responses to diverse conditions. Whether the latter will hold for all varieties of *P. aurelia* remains to be discovered. Four more varieties are under cultivation (reported in part in Sonneborn, 1943) in our laboratory and many more must exist in nature; but the sexual responses of these to diverse conditions have not yet been investigated.

SUMMARY

Among the 53 races of *P. aurelia* that have been investigated, four races (29, 32, 47 and 51) do not conjugate with any of the three previously described varieties. They constitute a fourth variety with two new interbreeding mating types, VII and VIII. Mating type VII occurs in all four of these races, but mating type VIII has appeared only in the two races 32 and 51.

The mating types VII and VIII give with each other the agglutinative mating reaction characteristic of Paramecium and proceed to conjugate. As in the other three varieties, agglutination and conjugation occur only when mixture is made between cultures of the two types that are neither well-fed nor starved, but are nearing the stage of nutritive exhaustion. Like variety 1, but unlike varieties 2 and 3, variety 4 shows no diurnal periodicity in sexual reactivity: cultures exposed to the natural alternation of daylight and night are capable of reacting sexually at any hour. Further, again like variety 1 and unlike varieties 2 and 3, variety 4 can react sexually throughout the range of temperatures from 9° to 36°, but not at 39°. At 16°, the sexual reactions are weak, leading to but a small proportion of conjugants. In mixtures made at higher temperatures and transferred at once to 9°, pairs begin to form but break apart without conjugating; however, if cultures are first adapted to 9° before they are mixed, a small proportion of true conjugation may occur at this temperature. In all these details, varieties 1 and 4 are alike; but they differ in behavior at the higher temperatures. The maximum optimum temperature for conjugation lies between 30° and 36° in variety 4, between 38° and 40° in variety 1. Thus at 36°, variety 1 gives a maximum sexual reaction, while variety 4 gives only 12 to 25 per cent of the optimum. Variety 4 shows a gradual falling off in sexual reactivity as temperature increases above the optimum, while variety 1 shows a sudden cessation of sexual reactivity at a temperature only 2° above the optimum.

It is thus possible to distinguish these four varieties of *P. aurelia* not only by their mating types, but also by the sexual responses to diverse conditions.

LITERATURE CITED

Sonneborn, T. M., 1938. Mating types in Paramecium aurelia: diverse conditions for mating in different stocks; occurrence, number and interrelations of the types. *Proc. Amer. Phil. Soc.*, **79**: 411–434.

Sonneborn, T. M., 1939. Paramecium aurelia: mating types and groups; lethal interactions; determination and inheritance. *Amer. Nat.*, **73**: 390–413.

Sonneborn, T. M., 1941. The effect of temperature on mating reactivity in Paramecium aurelia, variety 1. *Anat. Rec.* **81** (suppl): 131.

Sonneborn, T. M., 1943. More mating types and varieties in Paramecium aurelia. *Anat. Rec.*, **84**(4): 92.

HYBRIDIZATION AND SEASONAL SEGREGATION IN TWO RACES OF A BUTTERFLY OCCURRING TOGETHER IN TWO LOCALITIES

WILLIAM HOVANITZ

(*California Institute of Technology, Pasadena*)

The yellow and orange butterfly, *Colias chrysotheme*, exists in the form of two complexes known as the orange-race and the yellow-race (Hovanitz, 1943a; 1943b). These races have different geographical distributions but overlap over a tremendous territory from the Sierra-Cascade divide in western North America to the Atlantic ocean in the east and from southern Canada in the north through Mexico in the south (Hovanitz, 1943c). Each race usually occupies a different ecologic niche so that nearly pure populations of each may be found in this area as well as outside the zone of overlap. In certain localities, however, the same ecologic niche is partly occupied by both races, resulting in considerable hybridization between them.

Two localities where the races occupy the same niche for the most part were analyzed from 1941 to 1943 in order to study the behavior of each in relation to its environment, and to get an indication of the extent of hybridization between them. These places were at Mono Lake Valley, Mono County, California, and at Round Valley (near Bishop), Inyo County, California. Their positions are indicated on a map (Hovanitz, 1943d); they are just east of the Sierra Nevada in the western Great Basin.

The Seasonal Distribution of Adults

Orange butterflies are present throughout the entire warm season of the year at both Round Valley and Mono Lake. It is easier, however, to get a good sample in midsummer as compared with early spring or autumn. The abundance of orange adults apparently is at a minimum at each end of the growing season and at a maximum in midsummer.

The yellow butterflies at Mono Lake are more irregular in seasonal distribution than the orange (Fig. 1). The 1941 samples (Table I) show a high relative frequency of yellow to orange in May, and then a complete drop to none present at all in June. A rise to a second maximum in late July is apparent with a gradual drop again to none at all in September. Early in October there is a third maximum. This suggests three distinct broods per year at Mono Lake with an elapsed egg, larval and pupal development time of two months between each. This time compares with a development rate of three to four weeks at a constant laboratory temperature of 25° C. Mono Lake has a rather low air temperature, especially at night; in the day time, the direct radiation from the sun is the primary source of heat.

The 1942 samples at Mono Lake show much the same seasonal distribution.

The first adult flight was apparently not observed; it is probably very short in duration. The 1942 samples were obtained at monthly intervals rather than semimonthly as in 1941; therefore, the chance of missing a short adult flight is increased. The second and third broods of 1942 are to be found indicated in the figure a few weeks earlier than in the preceding year. As 1942 was a warmer year for Mono Lake than was 1941, an earlier start in larval development in the spring, with a consequent shift forward in the successive broods, would thus be expected.

The two 1940 samples at Mono Lake show no yellow butterflies present at all. Therefore, it would appear that they were obtained in a yellow interbrood period (Fig. 1).

The frequency at Round Valley does not follow this sequence of events (Fig. 1). Neither the 1941 nor the 1942 samples show any correlation with those of Mono Lake (Table I). This shows the complete lack of intermixing between the two places though they are only fifty miles apart. The 1941 Round Valley curve is high in late June (60 per cent yellow) and drops to a low in late July (25 per cent yellow). A rise occurs in mid-August (53 per cent), with a subsequent drop again the first of September (34 per cent), and then a last rise in early October (55 per cent). If these fluctuations represent successive broods not completely separated one from the other, then there are many more generations present per year at Round Valley than at Mono Lake. This would be expected considering the warmer climate at the former place (Round Valley is at an elevation of 4500 feet and Mono Lake at 6500 feet). Latitudinal differences in brood number per year parallel these altitudinal ones. There are three generations per year near Washington, D. C. (and Mono Lake), two generations near Hanover, N. H. (and

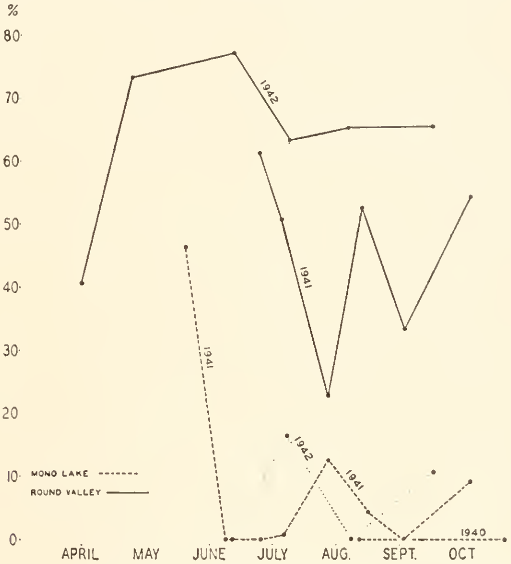


FIGURE 1. Frequency of yellow to orange butterflies at Mono Lake and Round Valley, California, throughout the season. Note the complete absence of yellow at certain times at Mono Lake as compared with Round Valley.

central British Columbia) and one generation in Alaska and Yukon Territory. At Round Valley there are probably four or more.

The 1942 samples are more extensive at Round Valley than are those of 1941 (Table I). There is a low of yellow (40 per cent) in early spring, rising to a high within the month of 75 per cent and later 79 per cent, with a rather constant frequency of 65 per cent yellow the remainder of the year. This curve shows little evidence of a series of broods or generations during the year. At the rather high temperatures prevailing in the valley during the summer (around 40° C. during the day, and fluctuating but not very cool at night), the succession of generations would be at about one month intervals. The samples were made at this interval of time, so it is quite possible that the sampling periods coincided with the periods of adult emergence. Were this the case, the results would show a rather constant seasonal frequency. On the other hand, it is possible that the variations in development rate between individuals owing to micro-temperature differences in the locality have completely eliminated the inter-brood population minima. This has been shown to be partially true for the second and third broods in the vicinity of Washington, D. C., as well as for New York state. In these

TABLE I

The frequency of the yellow-race as compared with the orange-race butterflies at Mono Lake and Round Valley, Calif. Standard errors are used in this and the other tables. The "many" is not included in the figures of totals but indicates the presence of yellow alone.

Date	Round Valley		Mono Lake	
	% yellow	N	% yellow	N
1940	—			
Aug. 11	—	—	0.0	105
Oct. 20	—	—	0.0	46
1941	50.50 ± 2.89	299	5.84 ± 0.63	1,387
May 4	many	many	—	—
May 19	—	—	46.27	75
June 8	—	—	0.0	91
June 24	61.19	134	0.0	70
July 5	50.82	61	0.89	678
July 26	22.92	48	12.66	237
Aug. 15	52.63	19	4.21	95
Sept. 2	33.33	15	0.0	20
Oct. 4	54.55	22	9.01	121
1942	65.96 ± 1.50	987	10.68 ± 0.88	1,236
April 1	40.82	49	—	—
April 25	74.55	110	—	—
June 12	77.14	140	0.0	many
July 7, 8	63.70	540	16.16	396
Aug. 6, 7	65.22	69	0.0	434
Sept. 16	65.82	79	10.67	406
1940–41–42	62.36 ± 1.35	1,286	7.68 ± 0.51	2,774

places, only the breaks between broods one and two are clearly defined by the absence of adults.

A higher frequency of yellow at Round Valley than at Mono Lake, at all times, is apparent (Fig. 1). Several factors combine to create this difference: (1) more larval food is present at Round Valley (Trifolium), (2) Round Valley is farther ecologically from the source of the migrant orange-race individuals (San Joaquin Valley), for these are more likely to stop in the mountain meadows than to proceed through the desert to Round Valley. The frequency of yellow is given as compared with orange. When the orange frequency goes down, the yellow will appear to rise in the curve. (3) The longer and warmer growing season at Round Valley gives more time for the resident population size to be built up. This has been shown elsewhere by the increased numbers of individuals in the second and third broods at Washington, D. C., and New York as compared with the first spring brood.

TABLE II

The frequency of intermediates in the mixed population of orange and yellow races of Colias chrysotheme *at Round Valley, California. (The total given in Table I does not include intermediates; hence, it is smaller than that given here.)*

Date	1941	N	Date	1942	N
	% intermediates			% intermediates	
May 4	—	many	April 1	10.91	55
June 24	14.65	157	April 25	7.56	119
July 5	11.59	69	June 12	14.15	163
July 26	9.43	53	July 7, 8	6.08	575
Aug. 15	32.14	28	Aug. 6, 7	6.76	74
Sept. 2	0.0	15	Sept. 16	12.22	90
Oct. 4	0.0	22	1942	8.27 ± 0.84	1,076
1941	13.08 ± 1.82	344	1941–42	9.44 ± 0.78	1,420

A higher frequency of yellows at Round Valley in 1942 as compared with the 1941 samples is also indicated. The latter samples were obtained in a mixed alfalfa-red clover field at the periphery of the large meadow which constitutes the primary ecologic niche for the yellow-race. The 1942 samples were made at a different field one mile from the latter (containing alfalfa, red-clover, white clover and native perennial clovers) in the center of the meadow. This field would be in the midst of the population for the yellow-race whereas the former field is on the periphery. For the migratory orange-race (Hovanitz, 1943d), no part of the meadow would constitute a population center. The higher frequency of yellow in 1942, therefore, can be accounted for by this change in position of the place sampled.

Hybridization Between the Races

Genetic data on crosses involving the races and on progeny from wild intermediates between the races indicate that crossing is easily possible and occurs frequently (Hovanitz, 1943b). Also, the indications are that there is no genetic sterility between the races. The F_1 is an exact intermediate of a light orange color; F_2 and backcrosses give the range of intermediates expected on a multiple factor distribution of genes.

The range of colors from the parental types through the intermediates is given in a range from yellow to orange of 1 to 10. From genetic results, it is known that grades 1 and 2 are pure parental types, breeding true for the yellow race. In the pure populations of orange race, there is a range of yellow to orange from 1 to 10 but from about 1 to 7 or 8 these are exceedingly rare (Hovanitz, 1943e). Therefore, grades 8 to 10 in the males and 7 to 10 in the females are considered as "parental types" for the Round Valley population. It is understood that grades 7 or 8 may be intermediates or that some lower grades may be parental types but that these will be insignificantly small.

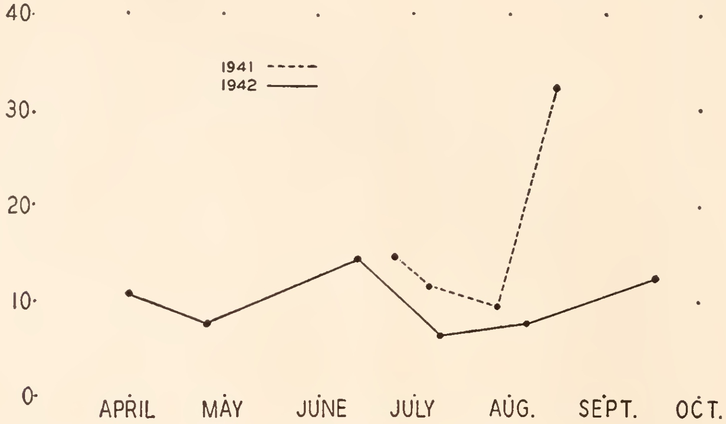


FIGURE 2. Frequency of intermediates between yellow and orange in the population at Round Valley, California, during the two years 1941–42.

On the basis of grades 3 through 7 in the males and 3 through 6 in the females, the frequency of intermediates in the Round Valley populations have been calculated (Table II). It is seen that there is but little seasonal change in the abundance of intermediates (Fig. 2). A high of 30 per cent in August 1941 is possibly a result of the small sample size. An average of about 10 per cent intermediates is usual.

Range of Wild Intermediates

The statistical consequences of continued interbreeding between the orange and yellow races should be a single race combining the characteristics of each parental type. But the two races have maintained their primary discreteness after more than 70 years of such interbreeding, and probably for many centuries (Hovanitz, 1943b; 1943c). Were the interbreeding only of very recent origin, the hybrid range would show a very high frequency of F_1 intermediates (grades 5 or 6) and a lower range of F_2, F_3 and backcross intermediates (grades 3–4, 7–8). The data on wild individuals (Fig. 3) do not show this higher frequency of F_1 to any great extent. The female curve may be masked by the normally low orange female grades. The male range shows a somewhat higher frequency of grade 5 than the other intermediates. The lack of the F_1 intermediates compared with F_n intermediates may be due to many factors of which a general lower viability seems to be the most likely (Hovanitz, 1943b).

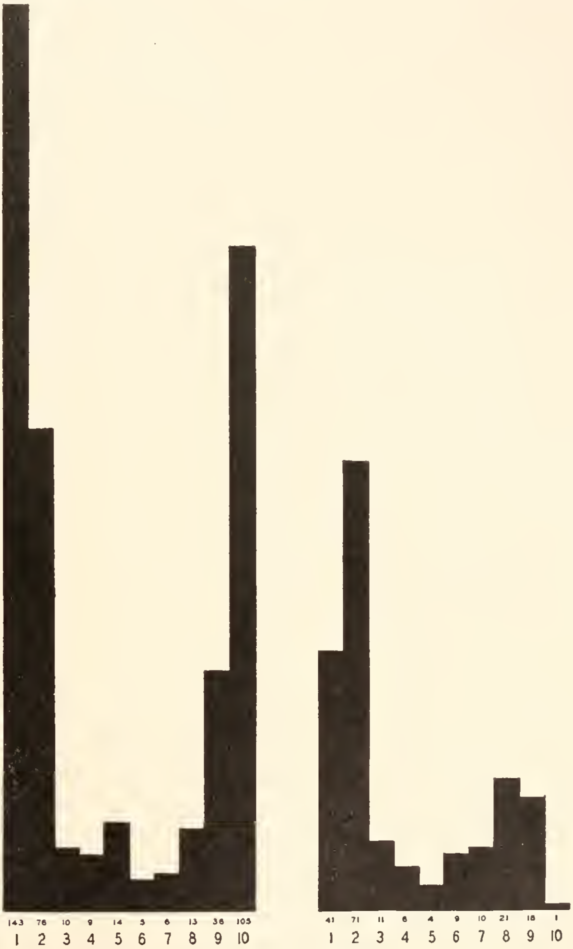


FIGURE 3. Histograms showing the range of variation in the intermediates between the orange and yellow races at Round Valley, California, 1941–42. Male on left and female on right. The smaller numbers represent the numbers of individuals in a given class and the larger represent the grade or class of intermediates.

DISCUSSION

The data on the existence of the two races of Colias living in the same locality suggest how ecologic and physiological differences can be maintained in units which may be called species. The races are not here called species for some genes are easily and often interexchanged (Hovanitz, 1943b). However, other genes are not effectively segregated in this way. This suggests that the significant gene complex characterizing each race and giving it individuality is not broken down in hybrid crosses.

Since the color difference separating the races is a multiple factor one and these factors are segregated independently of the basic complex, it might still be expected that a complete intermediate population would be produced, separated only by the non-visible basic complex. The reason for this lack of complete blending of characters probably lies in a combination of the following conditions:

(*a*) Sexual selection (Hovanitz, 1943b) may prevent sufficient intercrossing to be effective.

(*b*) Eggs genetically determined to be yellow-complex laid on alfalfa will later result in sterile adults or the subsequent larvae may die; also the reciprocal on red clover (Hovanitz, 1943; 1943b).

(*c*) The intermediates of all types are probably less viable than the parental types and many of them will be sterile on the food plant upon which they feed (Hovanitz, 1942; 1943b).

(*d*) The diapause associated with the one complex (Hovanitz, 1942, 1943b) tends to keep the races ecologically separated.

(*e*) The supplementary color genes of each normal type probably act better in unison with the basic complex than any intermediate segregation of genes.

(*f*) The different ecological niche occupied by the food plants necessary for each complex aids in preventing hybridization (Hovanitz, 1943c).

Summary

1. Two localities where the two races of *Colias chrysotheme* occur together are described (Mono Lake and Round Valley, Calif.).

2. In these places, the yellow-race has definite broods during the season. The orange race apparently does not.

3. The yellow-race has more seasonal generations when a population is at a lower elevation (Round Valley) than at a higher elevation (Mono Lake). This compares with latitudinal differences of the same type.

4. The two localities are 50 miles apart, but show no correlation in seasonal generations.

5. The yellow-race generations at the higher elevation are separated by interbrood periods with no adults. At the lower elevation, the generations merge one into the other.

6. Hybrid intermediates are present at one locality rather constantly at a frequency of about 10 per cent.

7. The range of color intermediates is not trimodal, but a U-shaped curve. This is probably due to a low viability of the F_1. A trimodal curve is expected under conditions of very recent hybridization and all intermediates with long-time hybridization.

8. Several reasons are given to account for the lack of complete blending between the races after years of hybridization.

Acknowledgments

This work was carried on through the encouragement of Professors T. H. Morgan and A. H. Sturtevant to whom the author is grateful.

LITERATURE CITED

Hovanitz, W., 1942. The biology of racial or species differences in Colias, *Bull. Ecol. Soc. Amer.*, **23**: 68 (Abstract).

Hovanitz, W., 1943a. The nomenclature of the Colias chrysotheme complex in North America. *American Museum Novitates* (in press).

Hovanitz, W., 1943b. Genetic data on the two races of Colias chrysotheme in North America and on a white form occurring in each. (Awaiting publication.)

Hovanitz, W., 1943c. The ecological significance of the color phases of Colias chrysotheme in North America. *Ecology* (in press).

Hovanitz, W., 1943d. The distribution of gene frequencies in wild populations of Colias. *Genetics* (in press).

Hovanitz, W., 1943e. The pattern elements of the North American Colias of the chrysotheme group. (Awaiting publication.)

SPECIES DIFFERENCES IN RATES OF OSMOTIC HEMOLYSIS WITHIN THE GENUS PEROMYSCUS *

HARRY P. LEVINE

(*Department of Zoology, University of Vermont, Burlington*)

Introduction

That definite species differences exist in the properties of the red cell membrane has been recognized at least since the studies of Rywosch in 1907. The possible significance of such specific differences in regard to zoological classification and animal identification has been pointed out by Jacobs (1931). Investigation of the rates of osmotic hemolysis in approximately 50 species of vertebrates led to the conclusion that "not only may individual species be identified but frequently unmistakable evidences of zoological relationship may be traced throughout a group of similar forms." In 1938 Jacobs and collaborators demonstrated striking differences in the permeability properties of the erythrocytes of the rat and mouse representing closely related genera. The purpose of the present investigation was to demonstrate measurable and consistent differences in the rates of hemolysis among a number of species within the genus Peromyscus.

Materials and Methods

The mice used in this investigation consisted of four species representing different degrees of taxonomic relationship (Miller, 1923) from widely separated geographical regions as follows:

Subgenus Haplomylomys Osgood
    P. eremicus fraterculus—La Jolla, California
Subgenus Peromyscus Gloger
    Species group—leucopus
        P. leucopus noveboracensis—Vermont; Moville, Iowa
        P. gossypinus palmarius—Sebring, Florida
    Species group—truei
        P. truei truei—Deadman Flat, Arizona

In addition, the guinea pig (*Cavia cobaya*) representing a distantly related rodent species was used for purposes of contrast.

Blood was obtained from each mouse under light ether anesthesia by cardiac puncture after the method of Hicks and Little (1931). About 0.5 cc. could be removed from a mouse without fatality. The blood was immediately expressed into a small beaker containing about 10 cc. of 0.9 per cent saline and defibrinated by stirring. The suspension was then washed down by centrifuge and the cells restored to the original blood volume with saline.

---

* Preliminary report presented at the 24th annual meeting of the American Society of Mammalogists in New York City, April 2, 1942.

The substances employed in the hemolysis studies were 0.3 molar solutions in distilled water of non-electrolytes including ethylene glycol, glycerol and erythritol representing progressively larger polyhydric alcohol molecules, and thiourea.

The method of determining rates of hemolysis was essentially that described by Jacobs (1930). To 5 cc. of one of the above solutions in a test tube in a water bath maintained at 20° C. was quickly added one drop of blood on a specially prepared plunger which simultaneously stirred the cells, producing an even suspension. With the aid of a stop-watch the time for 75 per cent hemolysis of the cells was determined by comparison with a standard suspension (one drop of the same blood in 20 cc. of saline) in a test tube adjacent to that containing the hemolysing suspension. This comparison was effected by means of a thin band of light viewed through the test tubes. Approximately 75 per cent hemolysis was attained when the band of light was visible in the hemolysing suspension to the same degree as in the standard. In practice the blood to be tested was so adjusted with saline that the band of light was just barely visible through the standard suspension since this offered the most easily recognized end point.

TABLE I

Species differences in rates of osmotic hemolysis

		Time in seconds for 75 per cent hemolysis at 20° C. in 0.3M											
		Ethylene glycol			Glycerol			Erythritol			Thiourea		
Species	No.	Low	High	Ave.	Low	High	Ave.	Low	High	Ave.	Low	High	Ave.
P. leucopus	17	4.7	6.4	5.6	7.0	12.3	9.5	20.6	49.0	31.8	10.7	15.3	13.3
P. gossypinus	14	5.6	7.8	7.1	15.0	28.5	22.1	47.0	195.0	110.0	13.3	21.7	19.4
P. truei	6	6.8	8.0	7.3	33.6	44.3	39.2	150.0	250.0	193.0	28.7	36.0	32.1
P. eremicus	15	5.6	6.7	6.0	31.0	58.5	44.3	150.0	465.0	259.0	16.8	28.5	23.3
Cavia cobaya	3	10.6	15.4	13.6	130.0	223.0	178.0	>30 hrs. <42 hrs.			116.0	143.0	126.0

In performing the experiments test tubes were carefully chosen for uniformity, standard suspensions were prepared as soon as the blood samples were obtained, and hemolysis rates were determined immediately. All tests were performed in duplicate whenever possible. Remaining portions of blood samples were kept in refrigeration storage at approximately 4° C. Except for certain storage experiments where pooled blood was used, hemolysis rates were obtained with erythrocytes from individual animals.

EXPERIMENTAL RESULTS

The method of determining rates of hemolysis as described above was very simple and apparently crude, but with proper care the results of tests performed in duplicate proved to be markedly consistent. Variation in duplicate measurements of the time for 75 per cent hemolysis of the red cells in any one of the solutions rarely exceeded 10 per cent and most often was less than 5 per cent. With practice, especially in preparing suitable standard suspensions, duplication was brought to within 2 per cent. It was reasonable to assume, therefore, that the differences in hemolysis times obtained here between one species and another represented true specific differences.

The times for 75 per cent hemolysis of the erythrocytes of the species investigated are summarized in Table I. Evidence of zoological relationship is readily apparent. When compared with the rate of hemolysis of guinea pig (Cavia) erythrocytes, the hemolysis rates of all the Peromyscus erythrocytes appear to be of the same order of magnitude. With erythritol, for example, the difference in hemolysis time between leucopus cells and eremicus cells (of the order 1 : 8) is small when compared with the difference between eremicus cells and guinea pig cells (1 : 540). On the other hand, consistent differences in hemolysis rates among the species within the genus are demonstrable. Leucopus cells are most readily hemolysed by each of the permeating substances; gossypinus cells are hemolysed at a somewhat slower rate. Generally truei and eremicus cells are hemolysed less rapidly than either leucopus or gossypinus cells. It is interesting to note in this regard that leucopus and gossypinus are placed taxonomically within the same species group.

TABLE II

Comparison of glycerol and thiourea times and G/T ratios of four species in the genus Peromycus (temperature 20° C.)

$$G/T\ ratio = \frac{\text{hemolysis time in glycerol}}{\text{hemolysis time in thiourea}}$$

	Time in seconds for 75 per cent hemolysis		
	0.3M Glycerol	0.3M Thiourea	G/T Ratio
P. leucopus	12.2	13.8	0.88
	8.1	13.7	0.59
	12.3	15.3	0.80
	7.0	10.7	0.65
	Ave. 9.5	13.3	0.71
P. gossypinus	28.5	21.4	1.33
	22.5	20.8	1.08
	24.4	21.7	1.12
	15.0	13.3	1.13
	Ave. 22.1	19.4	1.14
P. truei	44.3	33.2	1.33
	33.6	29.8	1.13
	43.4	36.0	1.21
	35.6	28.7	1.24
	Ave. 39.2	32.1	1.22
P. eremicus	58.5	27.3	2.11
	40.2	22.9	1.76
	54.6	27.5	1.98
	31.0	16.8	1.85
	Ave. 44.4	23.3	1.90

The rates of osmotic hemolysis in glycerol especially often reveal striking specific differences and sometimes offer evidence of relationship (Jacobs, 1931; 1938). From Table I it can be seen that all the Peromyscus red cells attain the condition of 75 per cent hemolysis in less than one minute. Yet the hemolysis times for the red cells of each species are apparently confined to definite limits within this time.

According to Jacobs and associates (1938), comparison of the rates of osmotic hemolysis in glycerol and thiourea within a species may provide an index for species identification. Table II records in the first two columns the hemolysis times in glycerol and in thiourea respectively for each species of mouse investigated. The figures in the third column (G/T ratio) are obtained by dividing the glycerol hemolysis time by the thiourea hemolysis time. The data have been selected to show the extent of variation found in each species. The average figure for each species is the arithmetic mean of the results for all individuals studied within each species. It is evident from the table that the glycerol/thiourea ratio is constant for each species within fairly narrow limits. Leucopus which has the lowest ratio and eremicus which has the highest ratio are readily separated from gossypinus and truei. Although the latter two species exhibit similar ratios, examination of the first two columns in Table II reveals that in the absolute times for hemolysis in glycerol and in thiourea they are readily differentiated.

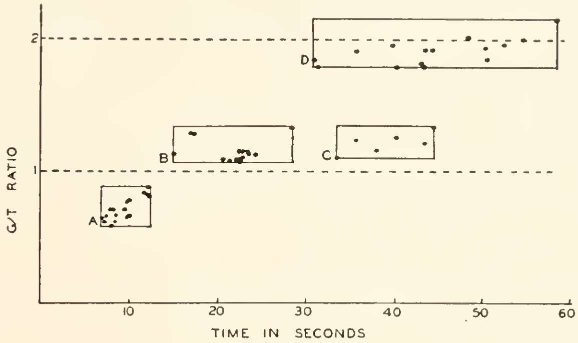


FIGURE 1. Species differentiation by osmotic hemolysis. Each dot represents an individual plotted along the abscissa in terms of the time for 75 per cent hemolysis in 0.3 molar glycerol at 20° C. and along the ordinate in terms of the G/T ratio. The hollow rectangles represent different species:

A = P. leucopus
B = P. gossypinus
C = P. truei
D = P. eremicus

Figure 1 records graphically the results which have been summarized in Table II. Each mouse investigated in the present study has been plotted with regard to erythrocyte hemolysis in glycerol (along the abscissa) and with regard to the glycerol/thiourea ratio (along the ordinate). The hollow rectangles enclose all the individuals within a species. This figure shows in a striking way that it may be possible to determine the species to which an individual belongs by the appropriate hemolysis tests. For example, at one stage in the course of these experiments a colleague kindly provided two blood samples without revealing the species from which they had been obtained. Hemolysis tests provided the following results:

	Time in seconds for 75 per cent hemolysis at 20° C.		
	0.3M glycerol	0.3M thiourea	G/T ratio
Mouse No. 1	12.1	15.0	0.80
Mouse No. 2	8.5	12.8	0.67

Both mice were correctly identified as leucopus.

Some evidence of zoological relationship is apparent in the glycerol/thiourea ratios obtained in this study. As can be noted in Table II, the ratios for leucopus, gossypinus and truei which are placed in the same taxonomic subgenus are all near one as a constant, while the ratio for eremicus which is placed in another subgenus is near two.

At the inception of this investigation some disconcerting variations in hemolysis times occurred within each species of Peromyscus. This led to an investigation of the effect of storage upon the rate of hemolysis of the red cells. In order to obtain a sufficient quantity for this purpose, it was necessary to use pooled blood of each Peromyscus species, whereas blood from individual guinea pigs was employed. Otherwise all blood samples were treated identically. Figure 2 shows the typical effect of storage upon the hemolysis rates of the Peromyscus and guinea pig red cells. Days in storage are plotted against the hemolysis time in glycerol. The red cells of each of the species within the genus Peromyscus show a marked and continued increase in hemolysis time upon storage while the red cells of the guinea pig show very little change during the same period of storage. The reason for this interesting storage effect has not yet been determined.

Discussion

Physiological and biochemical studies of blood have produced results both of broad evolutionary interest and also of value in the field of animal classification and identification. The evolutionary significance of results obtained from the studies of the osmotic pressures of blood (Scott, 1916) is well recognized. The extensive work of Reichert and Brown (1909) on the crystallography of hemoglobin among different species has provided convincing evidence of biochemical relationships among animals in general accord with the accepted taxonomic classification. The versatile and rapidly expanding field of systematic serology (see Boyden, 1942) has been employed on the one hand in the study of the possible origin of vertebrates (Wilhelmi, 1942), and on the other hand, in the investigation of the genetic basis for biochemical differences in the serum and blood cells of species and species-hybrids (Irwin and Cole, 1936; Irwin and Cumley, 1942).

The present investigation has revealed that consistent and measurable differences in the rates of hemolysis of the erythrocytes among very closely related species can be employed successfully to differentiate one species from another. Especially with regard to glycerol penetration, confirming observations by Jacobs, and with regard to the glycerol/thiourea ratio the results indicate zoological relationship in general agreement with the existing system of classification. Whether such agreement between morphological classification and rate of osmotic hemolysis will always hold among closely related species can be determined only by further investigation.

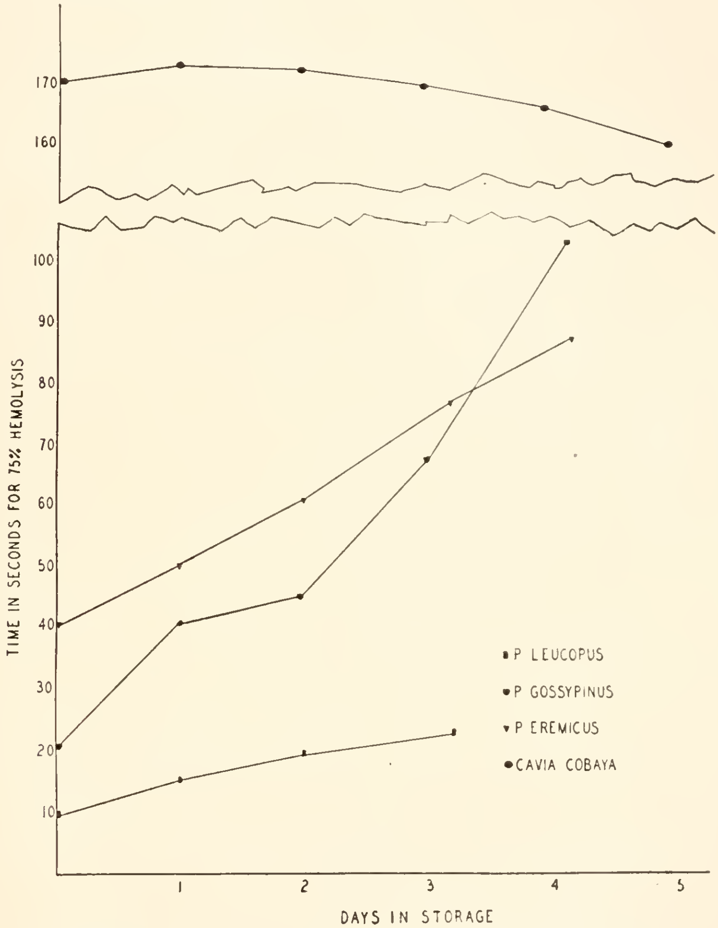


Figure 2. The effect of storage upon the rate of osmotic hemolysis (75 per cent) in 0.3 molar glycerol at 20° C. (Blood cells stored in 0.9 per cent NaCl at approximately 4° C.)

Preliminary studies on four offspring of a species-cross between leucopus and gossypinus indicate that these differences may be subject to genetic analysis although as yet the data are not sufficient for definite conclusions. Table III shows that in their hemolysis times in different substances the hybrid red cells are very similar to those of the leucopus parent stock while the values for the glycerol/thiourea ratio lie between those of the two parent stocks.

Specific differences in the properties of the cell membrane have introduced a complicating feature to the problem of cell permeability, yet an understanding of the nature of such specific differences may go far towards a better understanding of the factors determining the permeability of the cell membrane in general. In the meantime collection of further data on species differences in erythrocyte permeability will serve the useful purpose of developing a physiological means of animal identification.

The author is deeply indebted to Dr. Paul A. Moody who gave unstintedly of his mice and of his time when requested; to Dr. Lee R. Dice of the University of Michigan who provided some of the mice from which the present stock was originated; and especially to Dr. Merkel H. Jacobs of the University of Pennsylvania, under whose guidance the author became acquainted with the described hemolysis techniques at the Marine Biological Laboratory, at Woods Hole, Massachusetts. The author is further indebted to Dr. Jacobs for his kindness in reading the manuscript and for his valuable suggestions.

TABLE III

Comparison of hemolysis times and G/T ratios of a species hybrid and its parent stocks

Species	Time in seconds for 75 per cent hemolysis at 20° C. in 0.3M				G/T ratio
	Ethylene glycol	Glycerol	Erythritol	Thiourea	
**P. leucopus noveboracensis*	5.6	9.5	31.8	13.3	0.71
**P. gossypinus palmarius*	7.1	22.1	110.0	19.4	1.14
leucopus-gossypinus hybrids	4.8	8.0	28.0	8.6	0.93
	5.1	10.4	39.0	10.1	0.97
	5.2	10.2	34.0	10.5	1.03
	4.9	7.8	20.0	8.7	1.12

* Average of the species.

SUMMARY

The erythrocytes of four species of mice within the genus Peromyscus were studied with regard to their rates of osmotic hemolysis in ethylene glycol, glycerol, erythritol and thiourea. Consistent species differences in hemolysis times were demonstrated by which it was possible in the case of the individuals studied to identify each species with certainty. Evidence of zoological relationship was apparent in the results.

Refrigeration storage of Peromyscus erythrocytes resulted in progressively decreased rates of hemolysis. Storage of Cavia (guinea pig) erythrocytes had very little effect upon their rates of hemolysis.

LITERATURE CITED

BOYDEN, A., 1942. Systematic serology: A critical appreciation. *Physiol. Zool.*, **15**: 109–145.

HICKS, R. A., AND C. C. LITTLE, 1931. The blood relationships of four strains of mice. *Genetics*, **16**: 397–421.

IRWIN, M. R., AND L. J. COLE, 1936. Immunogenetic studies of species and species hybrids in doves, and the separation of species—specific substances in the backcross. *Jour. Exp. Zool.*, **73**: 85–108.

IRWIN, M. R., AND R. W. CUMLEY, 1942. Immunogenetic studies of species; qualitative differences in the serum of backcross progeny following a generic cross in birds. *Genetics*, **27**: 228–237.

JACOBS, M. H., 1930. Osmotic properties of the erythrocyte. I. A simple method for studying the rate of hemolysis. *Biol. Bull.*, **58**: 104–122.

JACOBS, M. H., 1931. Osmotic hemolysis and zoological classification. *Proc. Amer. Phil. Soc.*, **70**: 363–370.

JACOBS, M. H., H. N. GLASSMAN AND A. K. PARPART, 1938. Osmotic properties of the erythrocyte. IX. Differences in the permeability of the erythrocytes of two closely related species. *Jour. Cell. and Comp. Physiol.*, **11**: 479–494.

MILLER, G. S., JR., 1923. List of North American recent mammals. *U. S. Nat. Mus. Bull.* 128.

REICHERT, E. T., AND A. P. BROWN, 1909. The crystallography of hemoglobins. *Carnegie Inst. of Wash. Pub. No.* 116.

RYWOSCH, O., 1907. Vergleichende Untersuchungen über die Resistenz der Erythrocyten einiger Säugethiere gegen hämolytische Agentien. *Pflüger Archiv.*, **116**: 229–251.

SCOTT, G. G., 1916. The evolutionary significance of the osmotic pressure of the blood. *Amer. Nat.*, **50**: 641–663.

WILHELMI, R. W., 1942. The application of the precipitin technique to theories concerning the origin of the vertebrates. *Biol. Bull.*, **82**: 179–189.

GERMARIAL DIFFERENCES AND THE PRODUCTION OF APHID TYPES *

CHESTER A. LAWSON

(Department of Zoology, Michigan State College, East Lansing, Michigan)

INTRODUCTION

If germaria exercise any control over the development of differential characters in female aphids (Lawson, 1939; 1940) it is possible that they would give evidence of this control by exhibiting structural peculiarities correlated with the production of specific aphid types. To investigate this possibility the germaria of parthenogenetic females producing different aphid types were compared.

THE GERMARIA

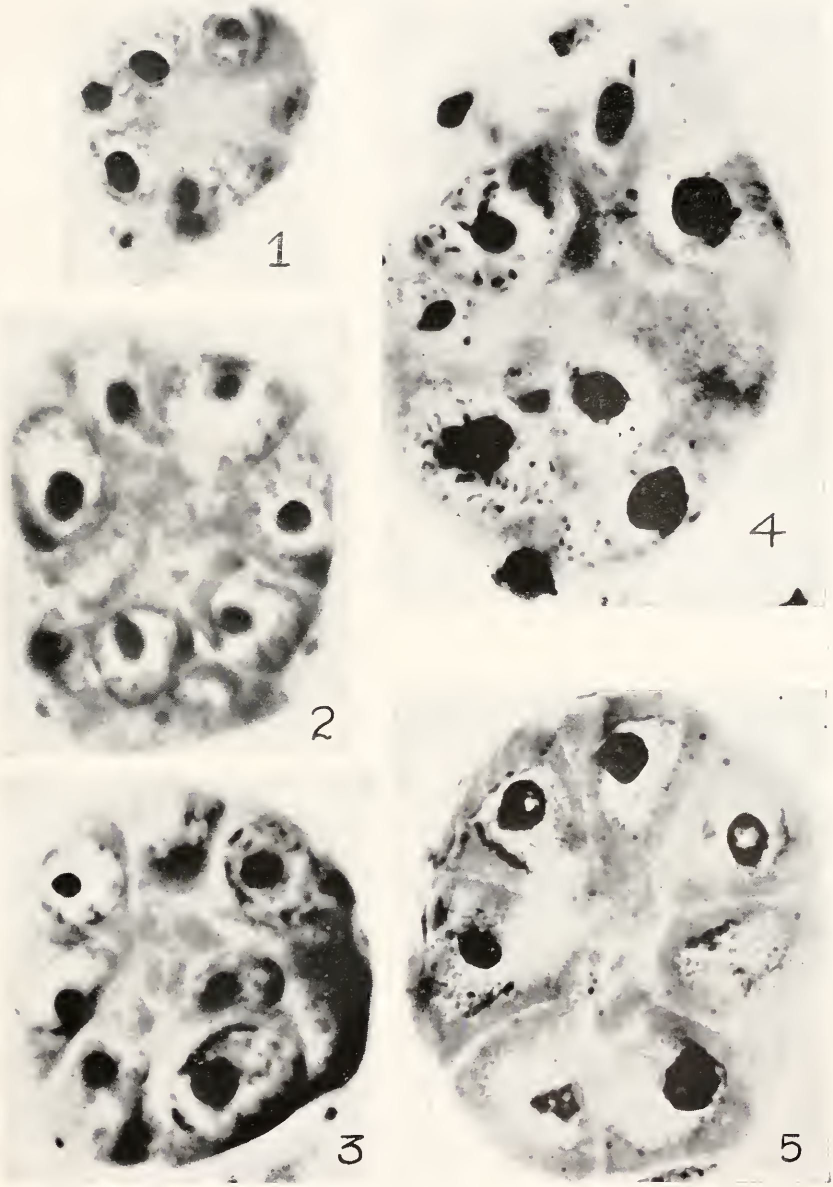
Each adult germarium contains two types of cells, nurse cells and germ cells. The nurse cells are larger than the germ cells and make up the bulk of the germarium, so if the germarium controls development it is possible that this control stems from the nurse cells. Their prominence in the germarium at least gives them first choice of the parts to be tested, so in this study the nurse cells only are compared.

The nurse cells of all parthenogenetic germaria are essentially alike (Figs. 1, 2, 3, 4). Each nurse cell is roughly pyramidal in shape (triangular in section) with the base at the periphery of the germarium and the apex in the center. The nucleus lies near the base of the pyramid and is covered on its outer edge and sides by a thin layer of cytoplasm. On the inner border of the nucleus the cytoplasm is thicker and extends inward toward the center of the germarium forming the apex of the pyramid. The cytoplasm seldom forms a sharp point in the center, for here it blends with the secreted substance found in the center of all germaria. The exact line of demarcation between cytoplasm and secreted material is difficult to see. The nuclei of all nurse cells are relatively large and each contains a large elliptical nuceolus and chromatin in the form of thin rods or prophase strands that are interconnected by a fine threadlike network.

In comparing the germaria of different aphid types, structural differences were sought that would serve to differentiate among them. Of several possible structural differences only one stands out with any consistency. This is a size difference. To test the reality of this apparent difference measurements were made and compared of the entire germarium and of individual nuclei within the nurse cells.

---

* Thanks are due to Professor W. D. Baten of the Mathematics Department who assisted with the calculations and to Professor C. P. Swanson of the Botany Department who made the photomicrographs. Part of this work was done at the Franz Theodore Stone Laboratory, Put-In-Bay, Ohio.



FIGURES 1–5. Cross-sections of adult aphid germaria. Figure 1. Winged parthenogenetic female producing gamic embryos (1455×). Figure 2. Winged parthenogenetic female producing parthenogenetic female embryos (1455×). Figure 3. Wingless parthenogenetic female producing parthenogenetic female embryos (1455×). Figure 4. Wingless parthenogenetic female producing male embryos (1455×). Figure 5. Adult gamic female (675×).

As each germarium is approximately spherical in shape its center cross section is circular or elliptical. The diameters of this cross-section were measured in micra and the area computed and this figure used to represent the size of the germarium. A better method of comparing the germaria would be to compare volumes. In order to calculate the volume of any one germarium it is necessary to have three diameters because very few of the germaria are perfect spheres. Two of these are easily measured on the center cross section. The third can be gotten by counting the number of cross sections of the germarium. However, no great reliance can be placed on a measurement arrived at in this manner. Each cross section is ten micra in thickness except the first and the last. These two vary from a fraction of one to ten micra, and as the actual thickness cannot be determined the third diameter has a possible error of twenty micra. Because of this error no confidence can be placed in the calculated volumes and it seems best to restrict the comparisons to the more accurately measureable center areas of the germaria. An occasional irregularity in the circumference of the cross sections introduces a source of error which is probably not great enough to discount major size differences, but may affect the results in comparison of minor differences.

Each aphid has nine or ten germaria and all of these that could be measured accurately were measured and all measurements from one type of aphid were grouped and treated statistically.

The means and standard deviations of the area of the center cross section of the adult germaria are given in Table I. The germaria of the male-producing wingless parthenogenetic females (Fig. 4) are larger than those of the wingless females producing parthenogenetic females (Fig. 3) and these in turn are larger than the germaria of winged females (Figs. 1 and 3). The differences between the means are statistically significant for all except the two winged types.

TABLE I

A comparison of the areas in square micra of germarial center section

Type of female	Contained embryos	n	Mean	Standard deviation
1. wingless parth.	males	103	1474±42	422±29
2. wingless parth.	parth. females	172	731±14	190±10
3. winged parth.	parth. females	126	599±16	178±11
4. winged parth.	gamic females	127	567±10	115±7

A difference between two means is considered significant when it is at least twice the standard error of the difference between means.

The Nurse Cell Nuclei

The nuclei of the nurse cells also were measured and compared. All the nuclei in any one germarium were not measured, but only those that were spherical. Many of the nuclei formed long ellipses or varied from the spherical unevenly. These nuclei were rejected in order to reduce the error of measurement and also to reduce the labor. If spherical nuclei only are used, one measurement, the diameter, is sufficient; and from this the volume can be calculated. This selection may introduce an error in the results if the shape of the fixed nucleus is correlated with its size, which is unlikely; or if an insufficient number of nuclei are measured in any one aphid type. It is believed that the number measured is sufficiently large to evade this source of error.

The means and standard deviations of nurse cell nuclear volumes in cubic micra are given in Tables II and III.

TABLE II

A comparison of nurse cell nuclear volume measured in cubic micra

Type of female	Embryos	n	Mean	Standard deviation
1. gamic		100	5180±314	3140±222
2. wingless parth.	males	205	953±28	402±20
3. wingless parth.	parth. females	408	326±7	140±4
4. winged parth.	parth. females	351	283±7	130±5
5. winged parth.	gamic females	200	151±5	64±3

TABLE III

Means and standard deviations in cubic micra of nurse cell nuclear volume of parthenogenetic females producing different types of parthenogenetic embryos

Type of aphid	Embryos	n	Mean	Standard deviation
1. wingless	winged and wingless	200	302±9	125±6
2. wingless	winged	208	348±10	148±7
3. winged	winged and wingless	351	283±7	130±5
4. winged	winged	198	279±8	129±6
5. winged	wingless	134	301±11	130±8

In Table II are listed the means and standard deviations of the five major aphid types. The differences in the mean nuclear volumes are all statistically significant. Thus the nurse cell nuclei of gamic female germaria (Fig. 5) are larger than any of the others, those of wingless females producing males (Fig. 4) are smaller than the gamic nuclei, but larger than any other parthenogenetic nurse cell nuclei. The wingless females producing parthenogenetic females (Fig. 3) have nurse cell nuclei that are smaller than the male-producing type but larger than those in winged females, while the winged female nurse cell nuclei are smaller than any of the others. There is also a nuclear size difference between the two types of winged females. The winged females producing gamic females (Fig. 1) have smaller nuclei than those producing parthenogenetic females (Fig. 2). In comparing Figures 1 to 5 it should be noted that the magnification of Figure 5 is approximately one-half that of Figures 1, 2, 3, 4.

The size differences shown by the nuclear measurements are in the same direction as those shown by the germarial measurements which suggest that the size of the entire germarium is due to the size of the nurse cells. One exception to this is seen in the two sets of measurements of the winged parthenogenetic females.

In comparing measurements of germarial center areas (Table I) the winged females producing parthenogenetic females and those producing gamic females are not significantly different. The means are different and direction of difference is the same as that of the nuclear size differences, but the difference is not statistically significant. In comparing the nuclear measurements of these same winged female types (Table II) a very large and significant difference appears.

One of the possible explanations is that no correlation exists between nuclear size and germarial size but rather between germarial size and nuclear (cell) number. If this is true the germaria of winged females producing gamic females should have almost twice as many nuclei as the germaria of winged females producing parthenogenetic females. A count revealed the same number in both (average 20 to 22). Another possibility is that there might be twice as much cytoplasm in each nurse cell within the gamic producing germaria, or that the material secreted by the nurse cells is excessive. These possible differences are not apparent on comparing the two types of germaria (Figs. 1 and 2) hence it is likely that there is some other explanation at present unknown. Also there remains the possibility that a difference may exist between the germarial areas of the two types of winged females (Table I) that is not shown in these calculations. The number of individuals used for computing the means of the germarial areas are one-half as many as are used in computing the means for nuclear volume of the same individuals (Table II). If n were doubled for the germarial areas a significant difference might appear.

In Table III are listed the means and standard deviations of parthenogenetic females that are producing parthenogenetic female offspring. The winged and wingless adults are classified according to whether they are producing either winged or wingless parthenogenetic female offspring or both.

The means are all about the same and none of the differences is statistically significant except for number 2 (wingless females producing winged embryos). This mean is significantly different from all in the table except number 5 (winged females producing wingless offspring). Thus except for one case no size difference is correlated with the production of parthenogenetic types and in this one case the difference is not great so it is possible that some factor other than type of offspring produced the difference.

If this interpretation is correct and there is no real size difference among the nurse cell nuclei in Table III a change must be made in the interpretation of Table II. In this table the mean nuclear sizes of number 3 (wingless parthenogenetic females producing parthenogenetic female embryos) and number 4 (winged parthenogenetic females producing parthenogenetic female embryos) are significantly different. However, the calculation of the mean of 326 ± 7 of number 3 of Table II includes the data under number 2 of Table III. If these data are eliminated from the calculations the mean becomes 302 ± 9 ($\sigma = 125 \pm 6$) and the difference disappears between this mean and that of the number 4, Table II (winged parthenogenetic females producing parthenogenetic female embryos). Thus the group of data in Table III that shows a questionable difference causes the difference between the winged and wingless parthenogenetic-producing females in Table II. Hence 3 and 4 in Table II probably are not different. There remain, however, the differences among the other types which are so large that their reality seems beyond doubt.

Germaria and Embryos of Winged-wingless Intermediates

A study of winged-wingless intermediates offers further evidence that the nurse cell nuclear volume is correlated with the type of offspring produced. In Table IV is presented a comparison of the mean nuclear volume of germarial nurse cells in individual winged-wingless intermediates and the type of embryos contained within the ovarioles of the intermediates. From this comparison it is evident that the intermediates having the smallest nuclear volume contain gamic female embryos within their ovarioles, and that as the nuclear volume becomes greater the embryos become parthenogenetic, then both parthenogenetic and male (in which the older embryos are parthenogenetic) then all male embryos and finally the intermediates having the largest nurse cell nuclear volume contain both male embryos and gamic eggs.

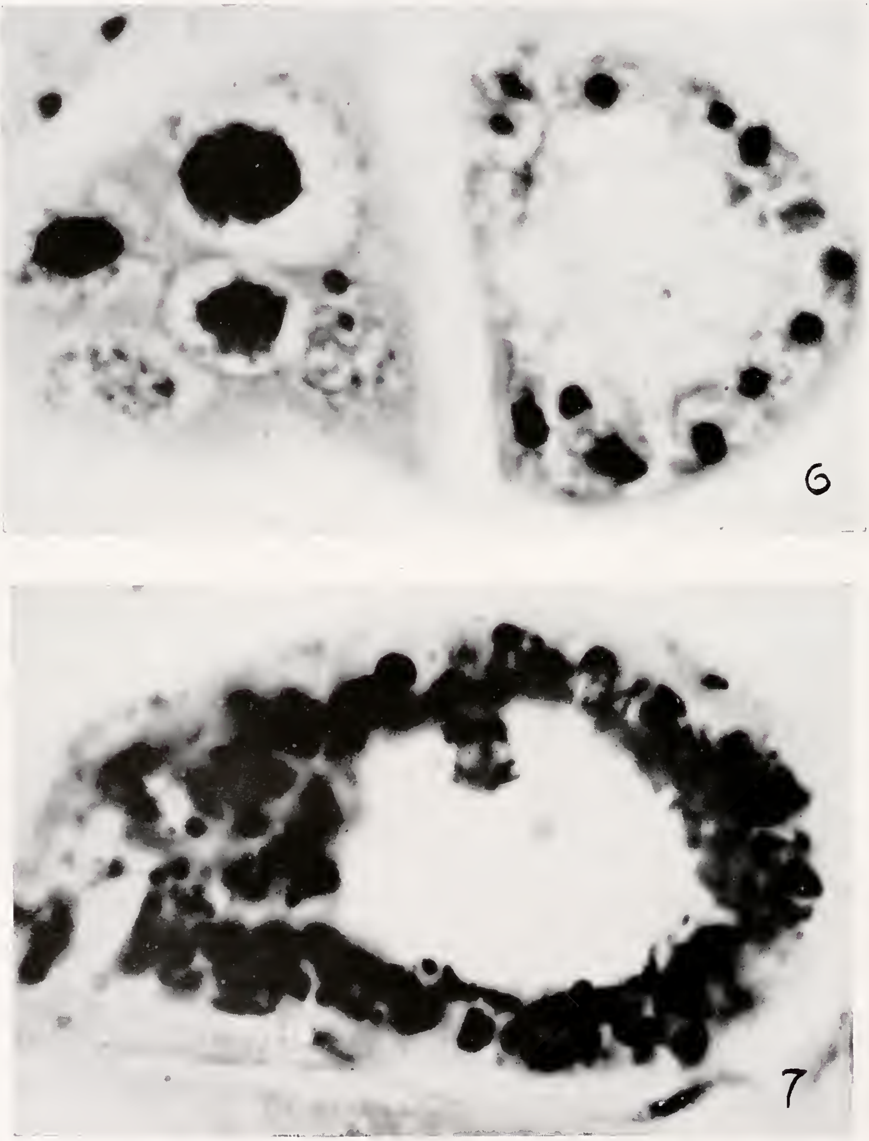
TABLE IV

A comparison of volumes in cubic micra of nurse cell nuclei in germaria of winged-wingless parthenogenetic female intermediates with the type of embryos contained in the vitellaria to which these germaria are attached

Mean nuclear volumes	Types of embryos
1. 57±9	Gamic female
2. 63±9	Gamic female
3. 132±10	Parthenogenetic female
4. 140±9	Parthenogenetic female
5. 157±8	Gamic female
6. 194±11	Parthenogenetic female
7. 235±14	Gamic and parthenogenetic female
8. 272±15	Parthenogenetic female
9. 353±31	Parthenogenetic female and male
10. 381±37	Parthenogenetic female and male
11. 383±23	Parthenogenetic female and male
12. 383±23	Parthenogenetic female
13. 486±58	Parthenogenetic female and male
14. 559±61	Parthenogenetic female and male
15. 732±93	Male
16. 804±73	Male and gamic egg
17. 930±82	Male

This correlation is not exact for intermediates 3 and 4, Table IV, contain parthenogenetic embryos while intermediate 5 has gamic embryos and also has a larger mean nuclear volume than either 3 or 4. Also intermediates 11 and 12 have the same nuclear volume, even though number 11 has both male and parthenogenetic female embryos, while number 12 has parthenogenetic embryos only. This irregularity may be due to the fact that all of the nuclei in any one intermediate could not be measured accurately, or it may be due to the effect of some unknown factor. In any case it seems reasonable to conclude that in winged-wingless intermediates the size of the nurse cell nuclei is correlated in general with the production of specific aphid types.

In one intermediate (17) there is an unusual germarium (Fig. 6) in which the nuclei are of two distinct sizes. The germarium is partly divided in half; one-half containing large nuclei (M = 2264 ± 390) the other half containing small nuclei (M = 445 ± 53). The appearance of two distinct sizes of nuclei within one germarium suggests that the size of any one nurse cell nucleus is determined by some factor within the germarium and perhaps within the individual nucleus itself. What this factor might be is entirely hypothetical; however, the nuclear size variation suggests polyploidy. No chromosome counts have been made as yet, but as the nuclei of gamic female germaria are filled with small rod-shaped chromosomes and are so much larger than any of the other types of nuclei it is probable that there is more chromatin in the gamic nuclei than in the others.



FIGURES 6–7. Figure 6. Abnormal germarium of a winged-wingless intermediate showing nuclei of two sizes (675×). Figure 7. Degenerate body (embryo?) found in ovariole of wingless parthenogenetic female producing males (675×).

Are all intermediates physiologically wingless?

Shull (1940) has suggested that adult winged-wingless intermediates are not physiologically intermediate but, rather, that they are wingless having progressed during development from a winged to a wingless condition. The structural characters become fixed in an intermediate condition during the transition and remain so during the life of the aphid, but the physiological nature of the individual continues changing until it is completely wingless. As a typical winged individual produces gamic females during the gamic phase of the cycle while a wingless female produces males, the physiological nature of the intermediates was determined by examining the type of offspring produced by them. Thus, if an intermediate produced males it was judged to be physiologically wingless. If it produced gamic females it was winged. Shull concluded that all winged-wingless intermediates are physiologically wingless.

An opposite conclusion is indicated by the evidence derived from the intermediates used in this study. These intermediates produced both male and gamic female embryos. Consequently some of them were physiologically winged and some wingless.

Degeneration in male-producing wingless females

Wingless females that are producing males not only have distinctive germaria but they also have degenerating cell masses within their ovarioles. The cell masses (Fig. 7) occur in the ovarioles at any position though they were observed most frequently at the end nearest the germarium. They are elliptical in longitudinal section and circular in cross section. A vacuolated center area is usually surrounded with a rim of densely staining pycnotic cells. What the degenerating bodies are is questionable but their elliptical shape is similar to young embryos, and furthermore, the rim of cells surrounding a vacuolated non-cellular center area is typical of young male blastulae. Therefore, it is tentatively concluded that the degenerating bodies are embryos that failed to continue development and are being resorbed. Why degenerating embryos should be characteristic of male-producing wingless females remains an open question.

CONCLUSION

A correlation between the size of the germaria and their nurse cell nuclei and the type of embryos produced seems established. Whether the germaria actually control production of aphid types is still unknown.

SUMMARY

The areas of the center cross-section of adult germaria of parthenogenetic female aphids producing different types of offspring were measured and compared. From this comparison it is evident that the center cross-sections of the germaria of male-producing wingless parthenogenetic females are larger than those of wingless females producing parthenogenetic females, and these in turn are larger than the cross-section of winged female germaria. All winged females have the same cross-sectional area whether they are producing parthenogenetic or gamic females.

A comparison of the volume of the nurse cell nuclei shows that the nuclei of gamic female germaria are larger than any of the others; those of wingless females producing males are smaller than the gamic nuclei, but larger than any other parthenogenetic nurse cell nuclei. The wingless and winged females producing parthenogenetic females have nurse cell nuclei of the same size, while the nurse cell nuclei of winged females producing gamic females are the smallest of all.

A correlation of the nurse cell nuclear volume of winged-wingless intermediates with the embryos contained in the ovarioles supports the thesis that size of nuclei and type of young produced are interdependent. Those intermediates that contained gamic embryos have the smallest nuclei; those with the next in nuclear size have both parthenogenetic and male embryos. The largest contain males only or males and gamic eggs.

LITERATURE CITED

Lawson, C. A., 1939. The significance of germaria in differentiation of ovarioles of female aphids. *Biol. Bull.*, **77**: 135–145.

Lawson, C. A., 1940. The developmental history of germaria in parthenogenetic female aphids. *Ohio. Jour. Sci.*, **40**: 74–81.

Shull, A. F., 1940. Adult intermediate-winged aphids not physiologically intermediate. *Genetics*, **25**: 287–298.

POLYDORA IN OYSTERS SUSPENDED IN THE WATER

VICTOR L. LOOSANOFF AND JAMES B. ENGLE

(Fish and Wildlife Service, Fishery Biological Laboratory, Milford, Connecticut)

Introduction

Among the numerous enemies of oysters the small Polychaete worms of the genus Polydora have long been considered as very destructive. It has been reported that sometimes these worms may be responsible for the complete disappearance of extensive oyster beds. Such depredations were described by Whitelegge (1890) and Roughley (1922, 1925) who were working in Australian waters, where Polydora caused a heavy mortality among the native oysters. Both authors identified the worm as *P. ciliata*. It is possible, however, that Whitelegge was mistaken in his identification of the species. According to Wilson (1928) "Whitelegge found the ova and larvae of a species of Polydora attached alongside the adults to the walls of their burrows in oyster shells at Newcastle, in New South Wales. He believed the species to be *Polydora ciliata* Johns, but his figure of the egg-sacs resembles more closely that given by Söderström for *Polydora ligni* Webster." If Whitelegge was actually mistaken then the destruction of the oysters in Australian waters should be attributed to at least two species of Polydora, namely, *P. ciliata* and *P. ligni*.

Several species of Polydora are common along our Atlantic Coast. Lunz (1940, 1941) found that approximately 40 per cent of the oysters of South Carolina waters are infested with *P. ciliata*. This author states in his latest paper that he now has evidence or reports of infestation throughout the entire range of distribution of the American oyster, *O. virginica*, in North America. Nelson and Stauber (1940) stated in a brief abstract that many oysters of New Jersey harbored *P. ligni* Webster. This appears to be the same species which, in the opinion of Wilson, Whitelegge was dealing with in Australia. Kavanagh (1940) found that the Japanese oyster, *O. gigas*, planted in Louisiana waters became infested with *P. ciliata*. Takahashi (1937) reported that *P. pacifica* was quite commonly present in the shells of the pearl oyster, *Pinctada margaritifera*.

Polydora or, as it is usually called, mud worm, is also known to infest shells of mollusks other than oysters. Lebour (1907) found that the mussels of the Northumberland beds of England were heavily infested with *P. ciliata*, and Field (1922) stated that the same species occurs in shells of the mussel, *M. edulis*, living in American waters.

Polydora usually gains entrance into the oyster while the worm is still in the larval stage, or when very young (Wilson, 1928; Roughley, 1925). Soon after entering the oyster the worm builds two mud tubes at right angles to the edge of the shell. The accumulated mud irritates the oyster tissue and the mollusk, in self protection, secretes a layer of shell material over the mud tubes. A description of the formation of mud blisters has already been given by Whitelegge (1890) and Lunz (1941) and need not be repeated here.

It has been the opinion of many investigators that the oysters infested with Polydora are usually very poor. If the infestation persists, they gradually begin to weaken and eventually succumb (Roughley, 1922; 1925). In some instances, as for example in Australia, it has been considered advisable to grow these mollusks on stones, logs or on specially constructed platforms, away from the bottom. Roughley (1922, 1925) believes that the method of keeping the oysters above the bottom mud is an effective means of preventing the infestation. It appears that Roughley's observations and data fully justify his conclusions in regard to the conditions existing in Australian waters. However, recent work of the authors carried on in Milford Harbor on the Connecticut side of Long Island Sound, showed that some of the habits of our species of Polydora and its effects on American oysters are somewhat different from those described for the Australian species, or previously ascribed to the mud worms common in American waters.

Description of P. Websteri Hartman

The mud worm found in the oysters of Milford Harbor was identified by Dr. Olga Hartman of Allan Hancock Foundation, The University of Southern California, as *Polydora websteri* Hartman, new name. In personal correspondence with the authors Dr. Hartman states that the original description of the worm, as *P. caeca*, was published by Webster, 1879. Since the description is faulty and misleading in all essential respects, it has little value for systematists. Dr. Hartman expresses an opinion that, unless caution is taken, the next reviser or systematist is almost certain to refer to our species as the European *P. ciliata*, since its morphological characters are closely akin to those of the latter. To avoid constant confusion of *Polydora websteri*, which at present is a systematically unknown species, with *P. ciliata* and some other species of Polydora that are known to be very numerous in eastern America, Dr. Hartman suggested that a description and the illustrations clearly indicating the characters of the worm should be given in this article. In accordance with the suggestion a description of *P. websteri* and the illustrations showing some of its morphological characters are offered here. Both the description and illustrations were prepared by Dr. Hartman.

"*Polydora websteri* Hartman

Polydora caeca Webster, 1879, Trans. Albany Inst., vol. 9, pp. 252–253, Figures 119–122 (not Oersted, 1843).

Polydora websteri Hartman (1942 MS on Beaufort Annelids).

The total length consists of about 105 segments and measures (preserved) 20 mm. long or shorter, but the body is usually much contracted and coiled up. The prostomium is clearly bifid at its anterior margin; it may lack eyes or there may be 3 or 4 weakly developed ones in trapezoidal arrangement; the prostomial parts, palpi omitted, are shown in dorsal (Figure 1, a) and ventral (Fig. 1, b) views. The first segment has a notopodial lobe but no notosetae, and the neuropodium is provided with a fascicle of slender setae. The second to fourth segments are biramous and have larger fascicles of notosetae and neurosetae with posterior lamellae. The fifth or modified segment is longer than the others and has, on either side, a dorsal fascicle of heavy yellow hooks with companion

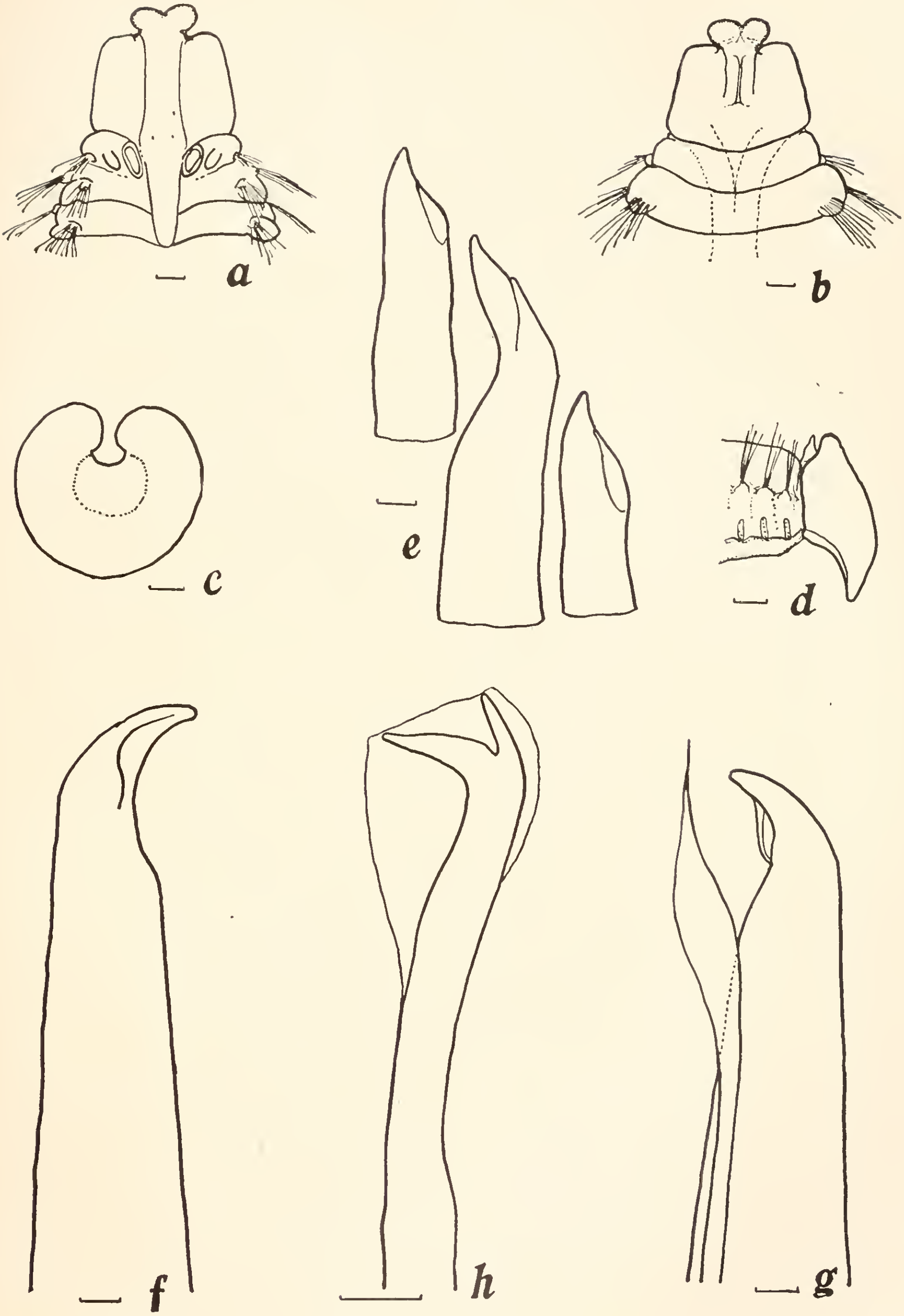


FIGURE 1. Showing certain morphological characters of *P. websteri*. Explanation in the text. (Courtesy of Dr. Olga Hartman.)

pennoned setae, and a ventral fascicle of 5 or 6 pointed setae. The seventh setiger has pointed setae in both fascicles. Hooded hooks are present from the neuropodium of the eighth setiger and continued posteriorly to the end. There are no specialized hooks in the last segments. The posterior end terminates in a flattened collarlike disk with a dorsal notch (Fig. 1, c in posterior view) considerably wider than the last few segments (Fig. 1, d in lateral view).

Branchiae, first present from the seventh setiger, are at first small but gradually enlarge to their full size in about 5 segments; they are continued through most of the body length but gradually decrease in size in the posterior fourth and are absent from the last 15 or 16 segments.

The heavy hooks of the fifth setiger number about 6 projecting ones in a fascicle; they are unique in that the falcate distal end has a hard, chitinous sheath around one side; various views are shown for projecting (Figs. 1, f, g) and embedded (Fig. 1, e) ones. The companion pennoned setae (Fig. 1, g) when perfect terminate in an acute point but some may be broken off and appear frayed at the distal ends. The hooded hooks number about 6 in a series in the middle of the body; they have 2 well developed teeth, the major one at a right angle to the shaft (Fig. 1, h). Tubes are fragile, constructed of silt and debris incorporated with mucus, and occur in calcareous shells.

The original description as *P. caeca* Webster is incomplete in some important details and erroneous in some others. The first segment has neurosetae, not notosetae; the pygidium is interrupted above, not below; the companion setae of the modified segment are pennoned, not capillary; the modified hooks of this segment are not merely falcate but have a sheath that extends some distance around it. There may be weakly developed eyespots.

P. websteri resembles *P. ciliata* (Johnston) (Fauvel, 1927, Faune de France, Vol. 16, p. 49) in some respects but the two differ in that the first has a prostomial caruncle that extends posteriorly to the end of the third setiger and the modified spines of the fifth setiger have a sheath around one side; in the second the prostomial caruncle extends posteriorly to the second setiger and the modified spines have an acute tooth in the concave part of the spine.

The single individual on which Webster's description was based is not known to exist. The collection on which the present description is based is deposited in the Allan Hancock Foundation of the University of Southern California. It was collected from vesicles on empty oyster shells, in the mouth of the Milford River, by Mr. J. B. Engle of the Milford Wildlife Laboratory. Since 1937 I have obtained this species in considerable number from Beaufort, North Carolina, Lemon Bay in southwestern Florida, and Virginia north to Connecticut. It may be widely distributed in intertidal zones of temperate North America.

(On the plate, the small scale near the label indicates 1 mm. for prostomium and pygidium and 0.1 mm. for setal structures.)"

The authors wish to express their appreciation to Dr. Olga Hartman for the identification of our species of the mud worm and for preparation of the description and the illustrations of the morphological characters of *P. websteri.*

OBSERVATIONS

These studies were begun in April, 1940, when five large groups of oysters, ranging from one to 5 years of age, were placed under observation in Milford

Harbor. In the summer of the same year another group, composed of individuals of the 1940 set, and thus being only a few weeks old, was added. Altogether over 1000 animals were used in the experiment. All these oysters were brought from the deep water beds of Long Island Sound, where Polydora is very uncommon. Examination of the oysters showed that only about 2 per cent of them had mud vesicles.

Oysters of each year-class were placed on separate, large, wire trays, suspended in the water from a float, which rose and fell with the tide. Even at low tide the trays were at least four feet above the bottom. The oysters remained suspended in the water until November 1, 1942. Thus, the experiment lasted $2\frac{1}{2}$ years, and covered two winter and three summer periods. At the end of the experiment a random sample consisting of 20 oysters was taken for examination from each year-class group. All the oysters were opened and the condition of their shells and meats noted.

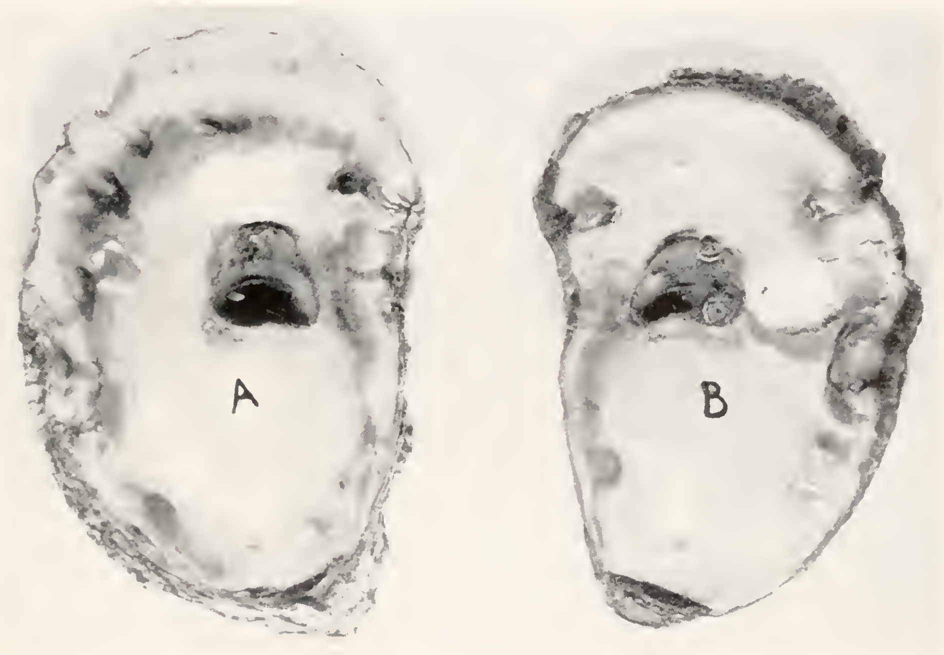


FIGURE 2. Shells of an oyster infested with *P. websteri*. *A*. Cup valve. *B*. Flat valve.

Examination of the shells showed that the oysters of all year-classes were heavily infested with *Polydora websteri* (Fig. 2). This was true even for those of the 1940 class which were but several weeks old when placed on the trays. The infestation was so heavy that in many instances separate mud vesicles could not be distinguished. Usually the combination of several vesicles formed large mud blisters. All the shells, with exception of one flat valve belonging to an oyster of the 1940 class, were infested. The class of 1935, comprised of the oldest oysters, had the greatest number of vesicles and blisters, while the youngest class had the least (Table I). However, since the shells of the older oysters offered much larger areas for infestation than those of the younger class, no direct relationship between the age of the animals and the degree of infestation could be assumed. Such a conception was further sustained by the lack of correlation between the age of the oysters and the degree of infestation in the other four year-classes (Table I). In general, the cup valves of the oysters contained more vesicles and blisters than the flat valves. This again cannot be regarded as significant because the surface of a cup valve is considerably larger in area than that of a flat one.

Careful examination of the character and positions of the mud vesicles, and the location of the characteristic double holes on the exterior of the shells through which the worms communicate with the outside, as well as studies of the cross-sections of the shells clearly indicated that the infestation was not confined exclusively to any one year within the experimental period. It was found, as the result of such examination, that the infestation with Polydora began during the summer of 1940 and continued until the end of the experiment.

TABLE I

Number of mud vesicles and blisters found in shells of oysters of different ages grown on the suspended trays and on the bottom. Each sample consisted of 20 oysters.

YEAR CLASS	TRAY OYSTERS				BOTTOM OYSTERS			
	Cup valve		Flat valve		Cup valve		Flat valve	
	vesicles	blisters	vesicles	blisters	vesicles	blisters	vesicles	blisters
1935	208	31	177	27	13	1	17	3
1936	136	3	103	2				
1937	188	20	123	17				
1938	208	39	111	24				
1939	189	33	156	28				
1940	126	20	81	8	3	0	4	0

While examining the shells of the oysters it was noted that in many instances of severe infestation as many as six or seven layers of blisters, superimposing one over the other, could be found over the same shell area. The worms occupying the lowest, and therefore the oldest, blisters were of a larger size than those of the upper ones. The occupants of the upper blisters were, as a rule, very small, indicating that they entered the shell only a short time before examination. Even under such apparently overcrowded conditions the majority of the worms were alive and, judging by the quantities of accumulated mud, very active.

Discovering an unusually heavy infestation of the tray oysters, it was decided to compare the degree of infestation of these animals with that of the mollusks living on the muddy bottom. For this, samples of 20 oysters of the 1935 and 1940 year-classes were taken from the bottom of Milford Harbor, in the area where the float with the suspended oysters was stationed during the experiment. Examination of the shells of the bottom oysters revealed that they were much less infested than those kept suspended in the trays. Many bottom oysters of the two year-classes were entirely free of mud worms. In the 1935 class, nine cup valves and seven flat ones bore no signs of infestation. The class of 1940 was in even better condition, because 17 cup and 16 flat valves were entirely free of vesicles or blisters (Table I).

In examining the condition of the oysters removed from the trays it was noted that, regardless of the very large number of mud worms infesting their shells, the oyster meats were in an excellent condition. They were unusually "fat," and large in size. They appeared much superior to those of the oysters usually grown in Milford Harbor. To verify this, a comparison was made of the experimental oysters and the animals taken from the bottom of Milford Harbor. It consisted in comparing the weight of the oyster meats in relation to their total weight. Each sample consisted of 20 oysters. The results obtained indicated that the animals suspended on the trays were much better than those collected from the bottom (Table II). This was especially true of the oysters of the 1935 year-class, where the bottom animals were found to be rather poor. The condition of the bottom oysters of this age-group was further substantiated by the observations made in connection with another series of experiments, dealing with seasonal changes in oysters in Milford Harbor. Samples of these oysters examined on November 15 and December 15, 1942, showed that on those dates the weight of their meats constituted 6.5 and 5.9 per cent of their total weight.

TABLE II

Average total weight and weight of meat, and per cent of meat of oysters of different ages grown on the suspended trays and on the bottom.

YEAR CLASS	TRAY OYSTERS			BOTTOM OYSTERS		
	Total weight	Weight of meat	Per cent of meat	Total weight	Weight of meat	Per cent of meat
1935	280.4	28.3	10.1	232.5	13.2	5.7
1936	216.2	22.1	10.2			
1937	202.0	21.9	10.8			
1938	154.2	17.2	11.1			
1939	122.1	15.0	12.3			
1940	73.1	10.1	13.7	21.8	2.4	11.0

On the basis of the above described observations the conclusion may be formed that a heavy infestation with *P. websteri* does not necessarily render the oysters poor. As was mentioned previously, the meats of heavily infested tray oysters were in an unusually good condition. Such a condition, of course, cannot be ascribed to commensalism with *P. websteri.* It indicates, nevertheless, that a heavy infestation of their shells does not prevent oysters from becoming "fat," provided other environmental conditions are favorable for the existence of the mollusks.

Regardless of the fact that the experimental oysters were suspended on the trays, away from the bottom, they were, nevertheless, covered with a very heavy layer of the deposit consisting of silt, mud and various dead and alive plankton forms. The thickness of this layer usually varied between 1/8 and 1/4 of an inch. Such accumulation of muddy substance was more than sufficient to supply the worms with all the mud needed for their activities. Therefore, no question could be raised whether or not there was enough mud to be carried by the worms for deposition between the shells of the oysters.

Indirectly, the experiments also provided an answer to the question of whether or not a severe infestation with *P. websteri* always causes a heavy mortality among the oysters affected. This answer is negative. For example, the most heavily infested year-class was that of 1935. In November 1941, this group consisted of 94 oysters. At the end of the experiment, in November 1942, 90 of these animals were still alive. Therefore, during the last year of the experiment, when infestation with the mud worms was presumably the heaviest, only four animals of the total number of 94 died. Thus, the mortality for the entire year amounted to only 4.3 per cent. This figure is considerably below that of the mortality of oysters of the same age but living under natural conditions, where a death-rate from 8 to 10 per cent is considered as normal.

It was also observed that a heavy infestation with mud worms did not interfere with the rapid growth of the oysters. All year-classes of suspended oysters, although heavily infested, showed a considerable increase in growth. The rate of growth greatly exceeded that of the less infested oysters living under natural conditions. The most noticeable difference was recorded in the case of the 1940 year-class, where at the end of two years, the average length of the suspended oysters was 79.2 mm. as compared with 63 mm. for the bottom oysters. Incidentally, our observations that the oysters kept off the bottom showed better growth are contradictory to those of Nelson (1921) who, on the basis of his experiments in which he also used wire trays, stated that "There was no appreciable difference in the rate of growth of oysters on the bottom from that of oysters on the platform above."

Discussion and Summary

It has been generally assumed that several species of Polychaete worms, such as *P. ciliata* and *P. ligni*, are very dangerous enemies of oysters interfering with their fattening and growth, and often causing a heavy mortality among them. It has also been stated that a heavy infestation with Polydora can be avoided if the oysters are grown away from the bottom mud. The method of growing oysters off the bottom is widely used in Australia.

Results of the experiments conducted for a period of 2½ years in Milford Harbor, Connecticut, indicate that in this body of water certain aspects of the behavior of at least one species of Polydora and its effects upon infested oysters are different from those observed in Australian waters, or ascribed to the mud worms of certain sections of our Atlantic Coast.

The Milford experiments have shown that mud worms, *Polydora websteri*, were found in much larger numbers in the shells of the oysters suspended in the water for a period of 2½ years than in those living on the muddy bottom. This indicates that in some areas along the Atlantic Coast of North America the suspension of oysters away from the bottom does not prevent, or eliminate, their infestation with the mud worms, *P. websteri*. Results of the experiments also point to the conclusion that the method of suspension may be regarded as providing sometime more favorable conditions for the mud worms to infest the oysters.

A complete explanation as to why the mud worms preferred the tray oysters to those on the bottom is still lacking. It may be suggested at this time, nevertheless, that the difference in salinity at the bottom, and in the zone where the oysters were suspended might have played an important part in the degree of infestation of the two groups. In Milford Harbor, which is a body of water affected by the river discharge and by inflow of salt water from the Sound, the salinity of the upper layers of the water is usually lower than that observed near the bottom. At times such differences are of considerable magnitude. For example, during the rainy period of 1942 occurring in August, the salinity of the surface layer varied between one and five parts per thousand, whereas at the bottom the salinity remained quite steadily above 25 parts per thousand. The fact that the heavily infested tray oysters were living in less salty water than those existing on the bottom may indicate that *P. websteri* prefers the water of considerably reduced salinity. Lunz (1941), on the basis of his observations in South Carolina, is also of the opinion that *P. ciliata* is more prevalent in water of low salinity.

The suggestion that *P. websteri* does not readily infest the oysters living in water of comparatively high salinity is substantiated by the authors' examination of oysters collected from Long Island Sound proper. During the summer of 1942 several thousand oysters of all ages were opened and examined. They were collected from many sections of the oyster-producing area of Connecticut. Very few oysters were found infested with Polydora. The salinity of the water of the area from which the samples were collected is usually above 26 parts per thousand (Loosanoff and Engle, 1940).

If certain species of Polydora, such as *P. websteri*, prefer water of low salinity, it is quite possible that several outbreaks of infestation of oysters with mud worms may be the result of prolonged rainy periods. In such cases large quantities of fresh water entering inshore shallow areas may considerably reduce the salinity of the water in which oyster beds are located, thus providing favorable conditions for the spreading of Polydora infestation. Experiments on the effects of various salinities upon the activities of Polydora, which are now being conducted by the authors, may throw additional light upon this very interesting and important subject.

Regardless of the heavy infestation with mud worms the meats of the tray oysters were in a far better condition than those of the mollusks living on the bottom. Their growth was also more rapid than that of the less infested animals of the same ages, but living under natural conditions. These two observations indicate that a heavy infestation with *P. websteri* does not necessarily interfere with the feeding and fattening of oysters, nor impair their growth. The apparent lack of ill effects upon the growth and fattening of oysters can be easily understood, if it is remembered that Polydora is not a parasite. Each worm remains in contact with the fleshy tissues of the oysters for a comparatively brief period. As soon as the mollusk covers the intruder and its mud tubes with a layer of shell material, the worm becomes isolated and cannot exert toxic effects upon the tissues of the oyster. It is probable, nevertheless, as Lunz (1941) indicated, that a large number of mud blisters within the shell may restrict the living space of the oyster, and that the animal may be forced to spend considerable energy in secreting the shell material for covering the mud worms. It is also possible that large quantities of mud accumulated by the worms on the bottom may render the environmental conditions unfavorable for the existence of the oysters and may even cause a heavy mortality among those mollusks (Roughley, 1922).

Milford experiments have also shown that a severe infestation with *P. websteri* did not cause a heavy mortality of the oysters. Our observations coincided with those of Lunz (1941) on *P. ciliata* who found that "In the five year period during which these pests have been under observation in South Carolina and other southern states, no high mortality has been found on oyster beds which could be attributed to the activities of Polydora."

LITERATURE CITED

Field, Irving A., 1922. Biology and economic value of the sea mussel, *Mytilus edulis*. *Bull. U. S. Bur. Fish.*, **38**: 128–259.

Kavanagh, L. D., 1940. Mud blisters in Japanese oysters imported to Louisiana. *Louisiana Conservation Review for Autumn*, **1940**: 31–34.

Lebour, M. V., 1907. The mussel-beds of Northumberland. *Northumberland Sea Fisheries Committee. Report on the Scientific Investigations for the year* **1906**: 28–46. New Castle-on-Tyne.

Loosanoff, Victor L., and James B. Engle, 1940. Spawning and setting of oysters in Long Island Sound in 1937, and discussion of the method for predicting the intensity and time of oyster setting. *Bull. U. S. Bur. Fish.*, **74**: 217–255.

Lunz, G. R., Jr., 1940. The Annelid worm, Polydora, as an oyster pest. *Science*, **92**: 310.

Lunz, G. R., Jr., 1941. Polydora, a pest in South Carolina oysters. *Journ. of the Elisha Mitchell Scientific Society*, **57**: 273–283.

Nelson, T. C., 1921. Report of the department of biology of New Jersey Agricultural College Experiment Station for the year ending June 30, 1920. New Jersey Agricultural Experiment Station, 1919–1920: 317–349.

Nelson, Thurlow C., and Leslie A. Stauber, 1940. Observations of some common Polychaetes on New Jersey oyster beds with special reference to Polydora. *Anat. Rec.*, **78**: 102.

Roughley, T. C., 1922. Oyster culture on the George's River, New South Wales. *Technical Education Series*, No. 25, Technological Museum, Sydney, 1–69.

Roughley, T. C., 1925. The story of the oyster. *Australian Museum Magazine*, **2**: 1–32.

Takahashi, Keizo, 1937. Notes on the polychaetous annelid *Polydora pacifica* n. sp. which bores holes in *Pinctada margaritifera* (Linné). *Palao Trop. Biol. Stat. Studies*, **1**: 155–167.

Whitelegge, T., 1890. Report on the worm disease affecting the oysters on the coast of New South Wales. *Records of the Australian Museum*, **1**: 41.

Wilson, Douglas, P., 1928. The larvae of *Polydora ciliata* Johnston and *Polydora hoplura* Claparède. *Jour. Mar. Biol. Ass'n N. S.*, **15**: 567–603.

THE ACTION OF ACETYLCHOLINE ON THE ISOLATED HEART OF VENUS MERCENARIA

ROBERT B. WAIT

(Biological Laboratories, Harvard University, Cambridge)

INTRODUCTION

The extraordinary sensitivity of the heart of the lamellibranch mollusc, *Venus mercenaria*, to acetylcholine was first reported by Prosser and Prosser (1937). Smith and Levin (1938) suggested the use of the isolated heart as a test object for acetylcholine and indicated its very much greater sensitivity to acetylcholine than to choline. The first detailed account of the responses of the *Venus* heart to acetylcholine and to nerve stimulation was by Prosser (1940). Prosser presented evidence that nervous inhibition of the heart is probably due to the release of acetylcholine at the terminations of nerve fibers from the visceral ganglion.

With the idea of using the isolated Venus heart for determining the acetylcholine content of tissues, when only small amounts are available, further experiments were carried out to ascertain the nature of the concentration-action curve and the imporatance of temperature control. The results will be reported briefly.

METHODS

Supplies of animals were obtained from a local market and stored dry at 5° C. until used. They ordinarily remained in a satisfactory condition for one to two weeks.

Certain minor changes in the method suggested by Prosser (1940) for isolating and perfusing the heart were made, hence the general procedure will be outlined. The soft parts were exposed dorsally by breaking and removing the umbos and hinge of the valves. The mantle and pericardium overlying the heart were cut away exposing the single, median ventricle and the laterally-disposed, thin-walled auricles. A thread was passed under each auricle and tied at the junction with the ventricle. The auricles were cut distal to the threads, also the anterior and posterior blood vessels and the intestine which passes through the heart. The isolated ventricle (which will be spoken of as the "heart") was placed in a bath with a capacity of 10 or 20 cc. when filled to the overflow arm. This was supplied with a common inlet-outlet tube at the bottom for perfusion fluid and an additional inlet for air, needed mainly for stirring since the oxygen requirements of the heart are low. This arrangement is shown in Figure 1. When temperature regulation was desired this chamber was submerged to the overflow arm in a water bath, the temperature of which could be kept constant or varied as desired. The heart was attached to a light heart lever counterweighted to 250 mg. and the beat recorded on a slow kymograph. The advantage of suspending the heart by the auricles is the avoidance of interference by the short length of intestine which passes through the longitudinal axis of the ventricle, the amplitude of beat being greater and more constant than when the heart is suspended by the anterior and posterior ends.

Since the blood of *Venus mercenaria* is very similar in composition to sea water (Cole, 1940) the latter was used as a perfusion fluid with quite satisfactory results. Glucose was added to the sea water in the proportion of 0.25 grams/liter. Isolated hearts have been kept beating for as long as three days at 15° C. During periods of washing the level of fluid in the bath was not allowed to drop below the level of the heart as such mechanical disturbance often causes temporary cessation of beat.

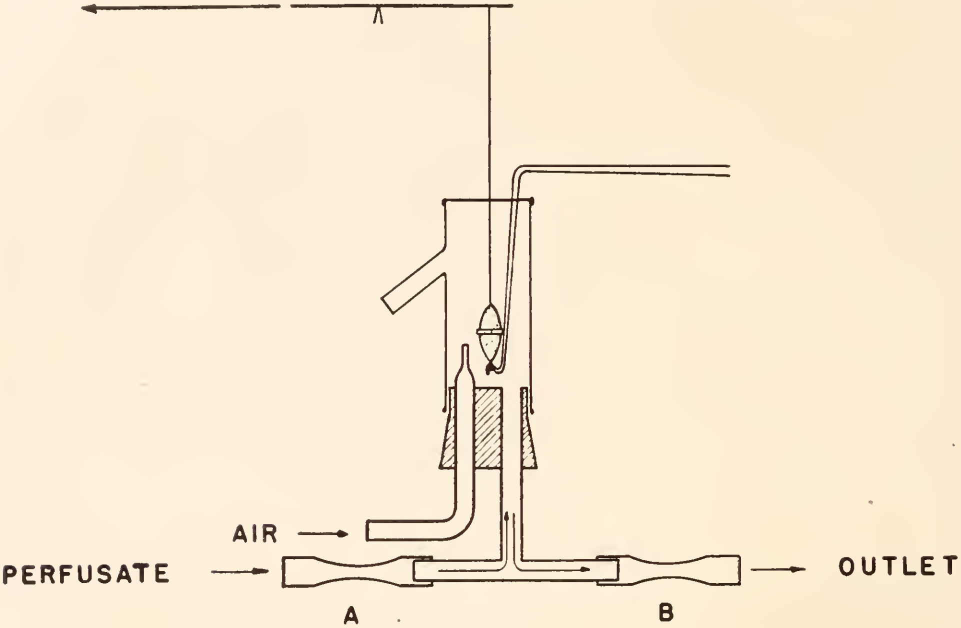


FIGURE 1. Perfusion chamber showing arrangement of air inlet and common inlet-outlet for perfusion fluid. Spring clamps at A and B, when opened and closed alternately, allow washing without complete removal of fluid from the chamber.

The acetylcholine used was in the form of the chloride and a stock solution was made up of 10^{-3} by weight of the alkaloid in 5 per cent NaH_2PO_4. This has a pH of approximately 4.0, at which acetylcholine is quite stable. The solution was sealed in small ampoules which were heated at 100° C. in a water bath for five minutes and then stored in a refrigerator until needed. Just before using, the stock acetylcholine was diluted with sea water so that a series of dilutions were at hand from which known amounts, up to one cc., when added to the bath, gave the desired concentration. The acetylcholine was added at the bottom of the bath by means of a hypodermic syringe with a long, small bore, glass tube bent at a right angle, in place of a needle. When the acetylcholine was added a corresponding volume of sea water was automatically displaced from the top of the bath before any appreciable mixing by the stream of air bubbles occurred.

In a given test the acetylcholine was left on the heart for one minute during which time the amplitude reached a new and nearly constant level. The heart was then washed with several changes of sea water and allowed a period of five minutes to recover its original amplitude before a second test was made. Measurement of the amplitude before and near the end of a given test allowed calculation of the amount of inhibition resulting from the action of the drug on the heart, and this was taken as a measure of effect.

Although eserine increases the sensitivity of the Venus heart to acetylcholine it was not used due to the fact that recovery between tests is more rapid in the non-eserinized heart.

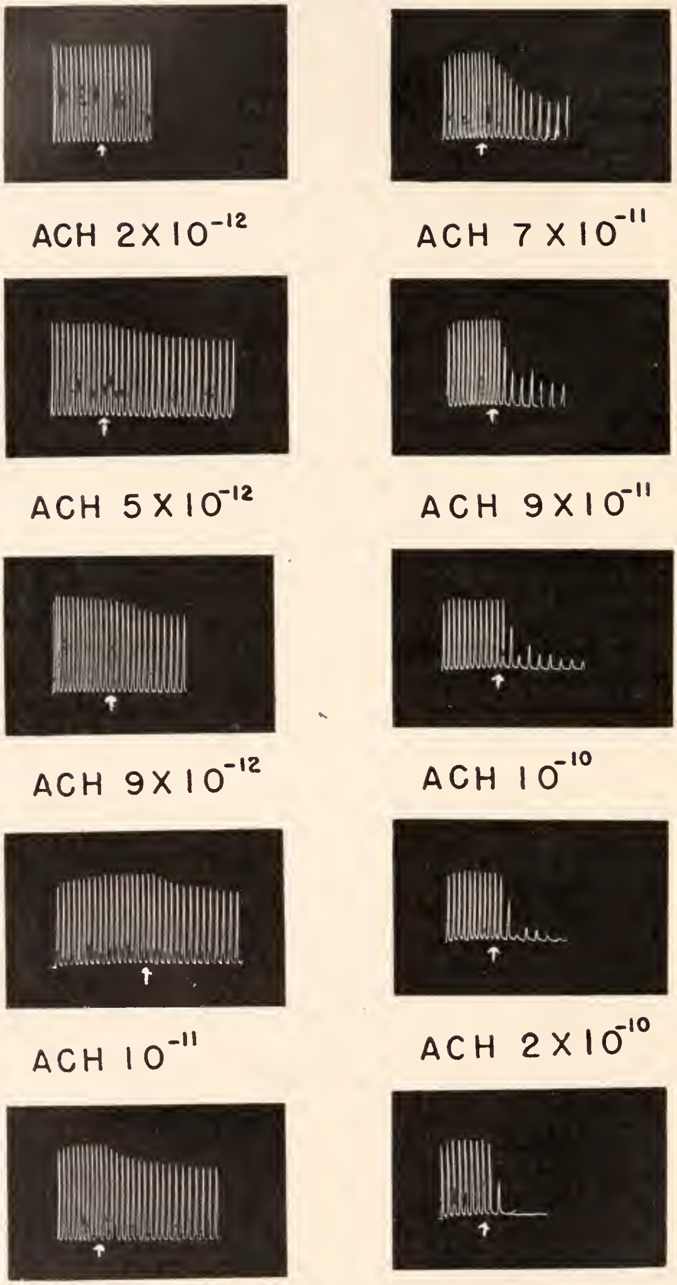
RESULTS

1. *The concentration-action curve for acetylcholine inhibition.*

Besides any theoretical significance in the quantitative relation between the concentration of a drug and its effect on a given biological system it is of practical importance in bioassay to know the nature of the concentration-action curve for the particular drug and the preparation being used. If it is not a straight line one can select that range where the response shows the greatest change with small differences in the concentration of the substance under investigation. Clark (1933) has pointed out that for most potent drugs such as acetylcholine, adrenaline, histamine and nicotine the concentration-action curves follow a hyperbola and that, depending on the kind of preparation and the recording system, responses are sometimes less accurately measured near the threshold and in other cases as they approach 100 per cent. It will be seen in the case of the Venus heart that the responses are most accurately determined in the vicinity of 50 per cent inhibition.

Concentration-action curves were obtained for 15 isolated Venus hearts. A series of sample records of the responses of one of these hearts is shown in Figure 2. From such records the per cent inhibition for each concentration could be measured and, in each case, when the results were plotted the curve was a hyperbola. Due allowance had to be made in some instances for the apparent complete inhibition of the heart before the flat portion of the curve was attained. This was due to the inertia in the recording system and the consequent failure of the record to show small residual movement.

In most preparations commonly used in the assay of acetylcholine, such as the isolated frog heart, the frog rectus abdominis and the dorsal muscle of the leech, the range over which graded action is obtained is from 1000 to 10,000 fold (Clark, 1933). In the case of the isolated lobster heart graded effects may be obtained over a 1,000,000 fold range (Welsh, 1942). In such cases it is customary to plot a measure of the effect against the logarithm of the concentration. Such curves are always S-shaped. When the amount of inhibition of the Venus hearts was plotted against the logarithm of the concentration most of the curves were such as is seen in Figure 3, which is that of a typical heart. This curve which was drawn to fit the points emphasizes the difficulty of making accurate measurements as the responses approach maximum. Since it is also difficult to determine accurately small amounts of inhibition it is obvious that in using the Venus heart for bioassay it is better to choose such concentrations of knowns and unknowns that the amount of inhibition produced is between 20 per cent and 80 per cent.



ACH 2×10^{-12}

ACH 5×10^{-12}

ACH 9×10^{-12}

ACH 10^{-11}

ACH 2×10^{-11}

ACH 7×10^{-11}

ACH 9×10^{-11}

ACH 10^{-10}

ACH 2×10^{-10}

ACH 2.5×10^{-10}

FIGURE 2. Sample kymograph records from a series on one heart showing graded action of acetylcholine over a range from threshold to complete inhibition.

2. *Effect of temperature on the response to acetylcholine.*

Some of the concentration-action curves were obtained in February, others as late as May. During February the concentrations of acetylcholine which produced a just measurable inhibition on different hearts were between 5 × 10^{-12} (1 : 5,000,000,000,000) and 5 × 10^{-11}. During May thresholds were found as high as 5 × 10^{-10}. This was at first thought to be evidence of a seasonal variation in sensitivity, although Prosser (1940) reported the highest sensitivity to occur in the spring. The experiments done thus far has been at room temperature and there was some evidence that the sensitivity of a heart was lower when the room temperature was abnormally high. Therefore a few experiments were performed to determine the effect of temperature on the response of the heart to acetylcholine. By means of a bath, with temperature control, the chamber containing the heart, and the perfusion fluid, could be maintained at any temperature between 5° and 35° C. The range over which hearts were observed to beat satisfactorily was somewhat less than this. Beginning in some cases at a low temperature and in others at a high, the concentration of acetylcholine was found which would produce a 50 per cent decrease in amplitude. The temperature was then increased or decreased and after a period of adaptation the concentration of acetylcholine necessary for 50 per cent inhibition was again de-

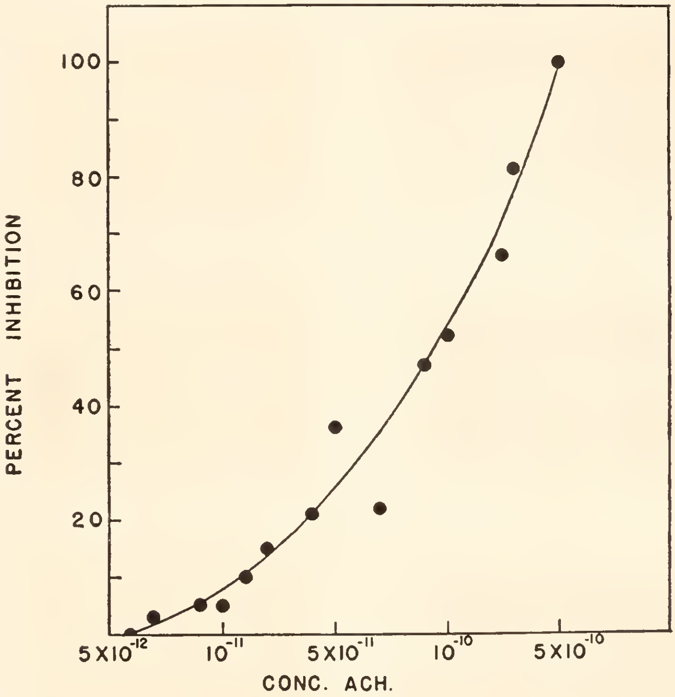


FIGURE 3. Data from a typical heart showing the decrease in amplitude (per cent inhibition) as a function of the concentration of acetylcholine (ACh).

termined. The results on three hearts, which had approximately the same threshold sensitivity at a given temperature, are shown in Figure 4. That there is a marked effect of temperature on the response of a given heart to acetylcholine is apparent. Approximately 100 times as much acetylcholine is required to produce 50 per cent inhibition at 30° C. as is required at 10° C. For this reason, and also from a consideration of the average environmental temperature of *Venus mercenaria*, it may be concluded that 15° C. is a satisfactory temperature at which to maintain the isolated venus heart for use in bioassay. If temperature control is not employed it is obviously necessary to perform a given set of assays at a nearly constant temperature.

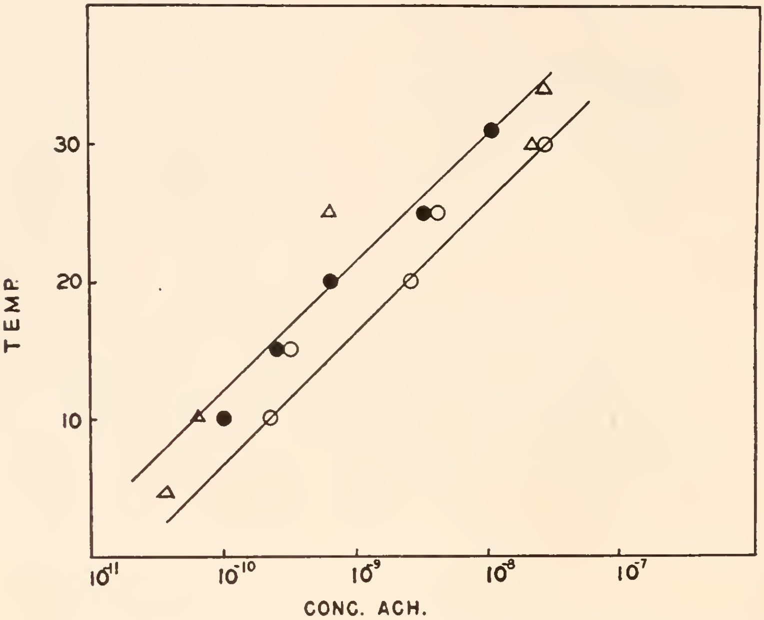


FIGURE 4. Data from three hearts, each represented by a different symbol, showing the concentration of acetylcholine (ACh) necessary to produce 50 per cent inhibition at different temperatures.

It is probable that the increase in the amount of acetylcholine required to produce a given amount of inhibition, as the temperature is raised, is due to the activation of the enzyme cholinesterase which destroys acetylcholine, and which is present in the Venus heart in small amounts (Jullien, et al, 1938; Smith and Glick, 1939).

CONCLUSIONS

This further study of the response of the isolated heart (ventricle preparation) of *Venus mercenaria* to acetylcholine provides information which confirms and extends that of Prosser (1940). Since the work was done with the practical viewpoint of eventual use of the preparation in assaying for acetylcholine in tissue extracts, little attention has been directed toward certain interesting theoretical problems. The demonstration that the concentration-action curve is a hyperbola, and recognition of the difficulty of recording beats of small amplitude, indicates that determination of acetylcholine values can most accurately be made when the concentrations are such as to produce between 20 and 80 per cent decrease in amplitude.

The importance of temperature control is evident. A heart which is relatively insensitive to acetylcholine at 25° to 30° C. becomes 100 times more responsive at 5° to 10° C. A temperature midway in this range has been found to preserve a beat of satisfactory amplitude and frequency for a convenient length of time (12 to 24 hours).

LITERATURE CITED

CLARK, A. J., 1933. The mode of action of drugs on cells. Edward Arnold and Co., London.

COLE, W. H., 1940. The composition of fluids and sera of some marine animals and of the sea water in which they live. *Jour. Gen. Physiol.*, **23**: 575–584.

JULLIEN, A., D. VINCENT, M. BOUCHET AND M. VIULLET, 1938. Observations sur l'acétylcholine et la choline-estérase du coeur des Mollusques. *Annales de Phys. et de Phys. Biol.*, **14**: 567–574.

PROSSER, C. L., 1940. Acetylcholine and nervous inhibition in the heart of Venus mercenaria. *Biol. Bull.*, **78**: 92–102.

PROSSER, C. L., AND H. B. PROSSER, 1937. The action of acetylcholine and of inhibitory nerves upon the heart of Venus (abstract). *Anat. Rec.*, **70**, Sup. 1: 112.

SMITH, C. C., AND D. GLICK, 1939. Some observations on cholinesterase in invertebrates (abstract). *Biol. Bull.*, **77**: 321–322.

SMITH, C. C., AND L. LEVIN, 1938. The use of the clam heart as a test object for acetylcholine (abstract). *Biol. Bull.*, **75**: 365.

WELSH, J. H., 1942. Chemical mediation in Crustaceans. IV. The action of acetylcholine on isolated hearts of Homarus and Carcinides. *Jour. Cell. and Comp. Physiol.*, **19**: 271–279.

Vol. 85, No. 2 October, 1943

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE HISTOGENESIS AND CYCLIC PHENOMENA OF THE SINUS GLAND AND X-ORGAN IN CRUSTACEA

ROBERT W. PYLE

(*Woods Hole Oceanographic Institution*[1] *and Department of Biology, Rensselaer Polytechnic Institute, Troy, New York*)

INTRODUCTION

In the decade that has followed Hanström's (1933, 1934) description of the sinus gland and X-organ in Crustacea a number of investigators, Sjögren (1934), Hanström (1937), and Ståhl (1938) have described them in detail. All of these studies have been concerned with a description of these glands as they appear in the adult animal. Since there has been little or no work done upon the histogenesis of either the sinus gland or X-organ, it is one of the objects of this paper to describe the histogenesis of both the sinus gland and X-organ in detail.

The endocrine activity of the sinus gland has been more or less well established through numerous studies in the past several years. As these are quite adequately and critically examined by Scharrer (1941) and Kleinholz (1942) there is no need to review the literature in detail. For further information of this nature one should examine those papers. Although extensive physiological studies have been made in relation to the endocrine function of the sinus gland, there have been no cytological studies made (except in Cambarus by Dethier 1942) to determine whether or not there are any evidences of cyclic phenomena in this gland. The role of the X-organ has been suggested, but no cytological studies have been made of it. Both the X-organ and sinus gland have been cytologically examined and the results of this study are reported herein.

METHODS AND MATERIALS

The histogenesis of the sinus gland and X-organ were studied in two species of Crustacea, *Homarus americanus* and *Pinnotheres maculatus*. The adults of these species and of *Cambarus virilis* were studied for cytological evidences of cyclic phenomena during the moulting period.

The eggs of Homarus were fixed in Carnoy-Lebrun: the first four stages after hatching were fixed in Zenker-formol and Bouin-Duboscq-Brasil, and the adult eye stalks (one week, 48 hours, six hours before, during, six hours, 48 hours, one,

---

[1] Contribution No. 326 from Woods Hole Oceanographic Institution.

one and one-half, four, six and thirteen months after moulting)[2] were fixed in Zenker, Zenker-formol and Bouin-Duboscq-Brasil. The eggs and first four stages after hatching were doubly imbedded in parlodion and paraffin and sectioned at five to nine micra. In some of the adults the exoskeleton of the eye stalk was decalcified and the whole eye stalk was doubly imbedded in parlodion and paraffin and sectioned at seven to 12 micra. In other adults the exoskeleton of the eye stalk was removed and the specimens were singly imbedded in paraffin. These were sectioned at seven to 15 micra.

The eggs, first zoea and adult stages (before, during, after and between moulting periods) of Pinnotheres were studied. The eggs and first zoea were fixed in Carnoy-Lebrun, Zenker-formol and Bouin-Duboscq-Brasil, and were doubly imbedded in parlodion and paraffin. Sections were cut at four to seven micra. The various stages of the adult were fixed in Zenker-formol and Bouin-Duboscq-Brasil, and were doubly and singly imbedded and sectioned at six to 12 micra.

The eye stalks of Cambarus were treated in the same way as those of Homarus; some were singly and some doubly imbedded. Sections were cut at seven and nine micra.

Serial sections were made of all specimens and these were stained with haemalum and eosin, Mallory's triple, Foot's (1933) and Lillie's modifications of the Masson trichrome stain, and the protargol method of Bodian (1937).

OBSERVATIONS

A. *Pinnotheres maculatus*

The X-organ is found in the embryo just before hatching (Figs. 1, 19) in that part of the eye which will become the median ventral side of the eye stalk in the

PLATE I[3]

The histogenesis of the sinus gland and the X-organ in *Pinnotheres maculatus*. All figures are oblique frontal sections of the right eye stalk. The neuropile of the optic ganglion is white and the ganglion cell layer is stippled.

FIGURE 1. Section of the late egg stage showing the position of the X-organ in relation to the structures of the optic ganglion. X—X-organ. S. G.—Sinus gland.

FIGURE 2. Section of the first adult stage showing the positions of the sinus gland and X-organ: both are distal to the medulla terminalis.

FIGURE 3. Section of the second adult stage. The sinus gland has begun to move distally, but the X-organ is found in the same general position as in earlier stages.

FIGURE 4. Section of the third adult stage. The sinus gland has advanced to a point between the medulla interna and medulla externa.

FIGURE 5. Section of the fourth adult stage. The sinus gland is lateral to the medulla externa at this stage.

FIGURE 6. Section of the fifth adult stage. The sinus gland now occupies a position between the medulla externa and lamina ganglionaris.

FIGURE 7. Section of the sixth adult stage. The sinus gland has advanced to a point that is distal to the lamina ganglionaris. In this as in previous stages the X-organ is found distal to the medulla terminalis.

---

[2] I am indebted to Dr. Charles J. Fish and the staff at the Wickford Hatcheries for the determination of the times in those specimens that were about to moult.

[3] All figures have been drawn with the aid of a micro-projection apparatus. All structures found between the hypodermis of the exoskeleton and the optic ganglia have been omitted for the sake of clarity.

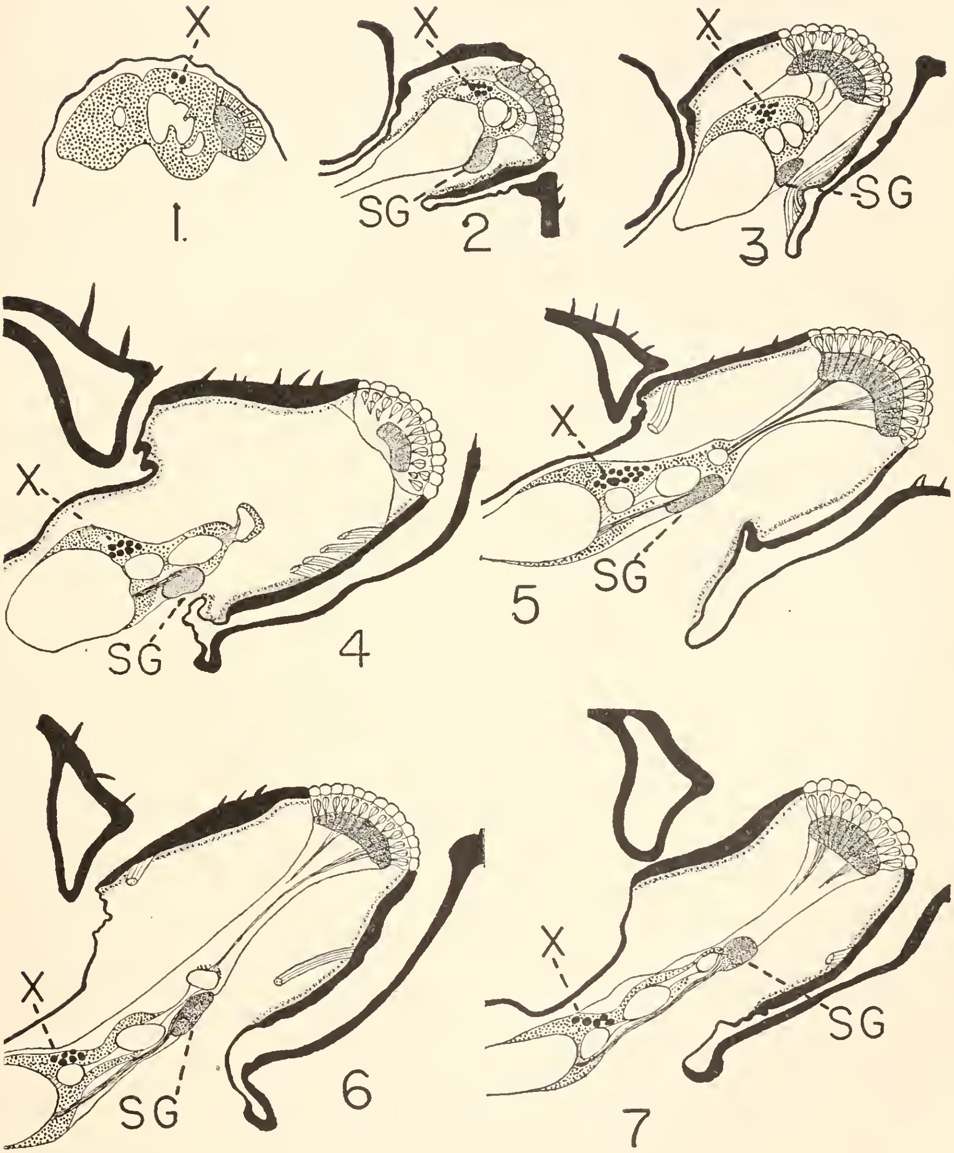

X
1.
X
SG
2
X
SG
3
X
SG
4
X
SG
5
X
SG
6
X
SG
7

PLATE I

first zoea. It occupies a position on the distal portion of the medulla terminalis and is almost entirely surrounded by the cells of the optic ganglia. There is also found, usually between the X-organ and the medulla terminalis, an area which is devoid of cells; this appears in serial sections as a hole. As far as can be determined from cytological preparations the cells of the X-organ are very similar to those found in the ganglion cell layer. They are probably derived from the same embryonic source and later become differentiated into X-organ cells. There is no evidence of any nerve fiber connections with the medulla terminalis. The nuclei are of the same size, shape and appearance as the nuclei of the ganglia cells. There is more cytoplasm present than in the ganglia cells; it is non-granular and clear. The X-organ is an integral part of the ganglia cell layer and is not set apart from it by a connective tissue sheath.

The secretory products of the X-organ are large rounded masses which exhibit concentric rings; this seems to indicate that the secretions have been laid down at different intervals. These secretory products always give a basophilic reaction when stained; they are blue after aniline blue and are structurally very similar to those found in the X-organ of Homarus. There is no evidence of any cyclic phenomena in the egg stage as the secretions have the same characteristic appearance in all specimens.

There is no evidence that the sinus gland has been developed by the late egg stage or the first zoeal stage. Unfortunately, conditions existing at Woods Hole last summer did not permit obtaining the intermediate forms between the first zoeal and the first adult stage so that these could not be studied. Attempts to raise them beyond the first zoeal stage were fruitless.

The adults may be grouped into six categories or stages which correspond to the moults. This is comparable to the five moults found in *Pinnotheres pisum* by Atkins (1926). In all stages of the adult (Figs. 2–7, 21) the X-organ is found in the same relative position that it occupies in the egg stage. The number of cells of which it is composed increases after each moult, but no mitoses were observed at any time. The cells are larger than the ganglia cells which surround them. They are wedge-shaped and are grouped in such a way as to remind one (when examining serial sections) of a pie that has been cut; the nuclei are found around the periphery and each cell becomes narrower as its cytoplasm extends toward the center of the X-organ. As the cytoplasm becomes filled with the secretory products the nucleus is pushed more and more toward the periphery

---

PLATE II [4]

The histogenesis of the sinus gland and X-organ in *Homarus americanus*. All figures are oblique frontal sections; figures 8, 9, and 11 are of the left eye stalk, and figure 10 is of the right eye stalk. The neuropile of the optic ganglion is white and the ganglion cell layer is stippled.

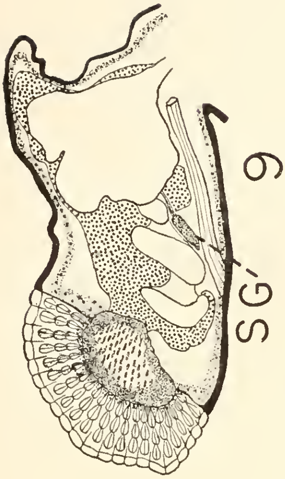
FIGURE 8. Section of the late egg stage showing the position of the X-organ.

FIGURE 9. Section of the third stage after hatching. The sinus gland is seen as a thin structure on the proximal side of the medulla externa.

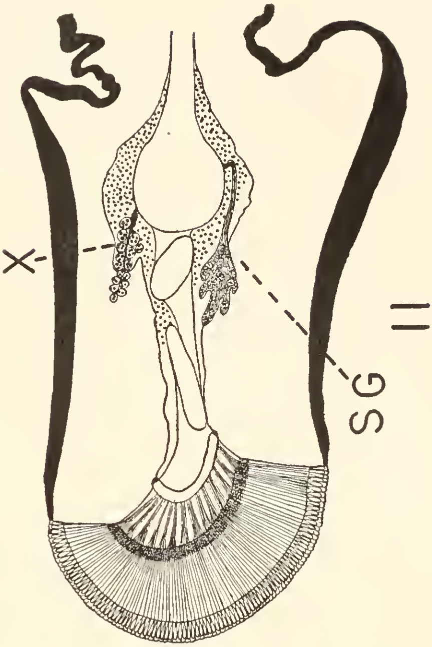
FIGURE 10. Section of the fourth stage after hatching. The sinus gland still occupies a position on the proximal side of the medulla externa. The X-organ extends to the hypodermis of the exoskeleton where the eye papilla is found.

FIGURE 11. Section of the adult stage. Both the X-organ and sinus gland, on opposite sides of the eye stalk, are seen extending beyond the limits of the neurilemma which surrounds the optic ganglion.

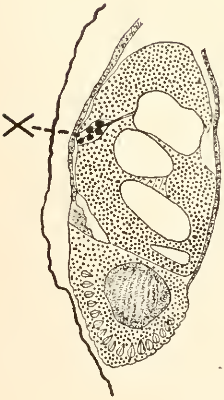
[4] See footnote 3.



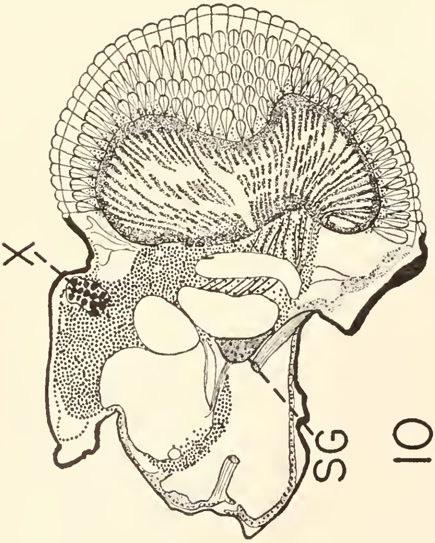
9



11



8



10

PLATE II

of the cell. In no stage is there any evidence that the X-organ has a nerve fiber tract which extends from it to the medulla terminalis. Cytologically there is no evidence of any cyclic phenomena associated with the secretion processes of the X-organ at any time. It always exhibits the same basophilic reaction regardless of whether it is fixed before, during, after or between moulting periods.

The sinus gland is well developed by the time the animal reaches the first adult stage (Fig. 2). It is at this time that it enters the mussel, *Mytilus edulis*, and begins its parasitic form of existence. The most remarkable feature in the subsequent development of the sinus gland is its change of position in relation to the structures of the optic ganglia. In the first stage (Fig. 2) it is found closely appressed to the medulla terminalis; in the second stage (Fig. 3) it has begun to move away from the medulla terminalis. In each successive stage it advances farther toward the distal portion of the eye stalk. In the third stage (Fig. 4) it occupies a position between the medulla interna and the medulla externa, in the fourth stage (Fig. 5) alongside the medulla externa, in the fifth stage (Fig. 6, 21) between the medulla externa and lamina ganglionaris, and in the sixth stage (Fig. 7) it has advanced to a point that is distal to the lamina ganglionaris.

In all stages the sinus gland is found on the dorso-lateral side of the eye stalk. There are very few nuclei in it (Fig. 23) and these bear such a close resemblance to those of the neurilemma, which is continuous with the sinus gland, that one might well consider the sinus gland a modification of the neurilemma (cf.

PLATE III[5]

Microphotographs to show the cyclic phenomena in the sinus gland of *Homarus americanus* and *Cambarus virilis*. All photographs are × 725 reduced about 35 percent, and are of materials fixed in Bouin-Duboscq-Brasil and stained with Foot's modification of the Masson trichrome stain.

FIGURE 12. *Homarus americanus*. A portion of the edge of one lobule of the sinus gland of a specimen fixed forty eight hours before moulting and sectioned at twelve micra. The brilliantly staining acidophilic secretory products are seen as dark irregularly shaped masses. This and succeeding figures show the loose network of connective tissue which constitutes the framework of the gland and the very few scattered nuclei.

FIGURE 13. *Homarus americanus*. A portion of the edge of one lobule of the sinus gland of a specimen fixed six hours after moulting and sectioned at ten micra. The bulk of the secretory masses are only slightly acidophilic and appear less dark in the photograph.

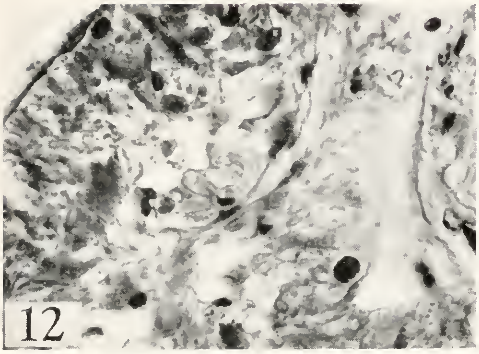
FIGURE 14. *Homarus americanus*. A portion of the edge of one lobule of the sinus gland of a specimen fixed six months after moulting and sectioned at twelve micra. The secretory material is reduced in quantity and stains in a slightly acidophilic manner.

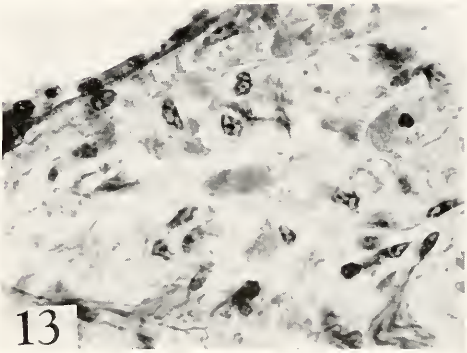
FIGURE 15. *Cambarus virilis*. A portion of the sinus gland of a specimen fixed before moulting and sectioned at nine micra. The numerous brilliantly acidophilic secretory masses are seen as dark masses hung upon the connective tissue framework of the gland. The blood sinus shows as clear areas.

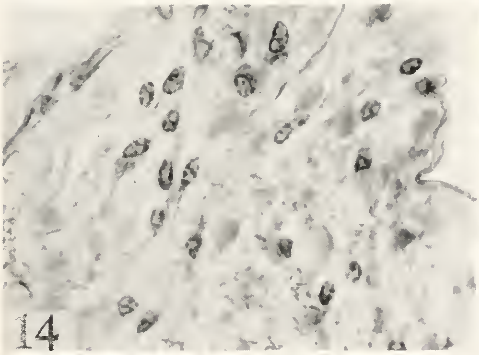
FIGURE 16. *Cambarus virilis*. A portion of the sinus gland of a specimen fixed after moulting and sectioned at nine micra. The secretory products are conspicuous by their absence. The blood sinuses are filled with blood. The nuclei are scattered at random in the loosely arranged connective tissue.

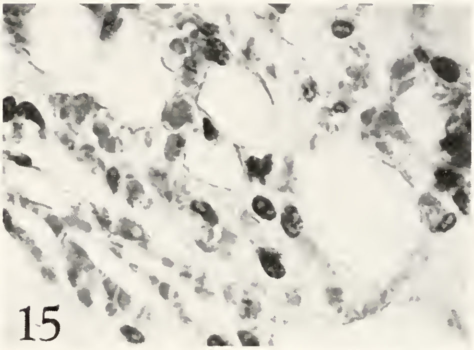
FIGURE 17. *Cambarus virilis*. A portion of the sinus gland of a specimen fixed in December and sectioned at nine micra. The majority of the secretory products present are acidophilic. The blood sinuses appear as clear areas.

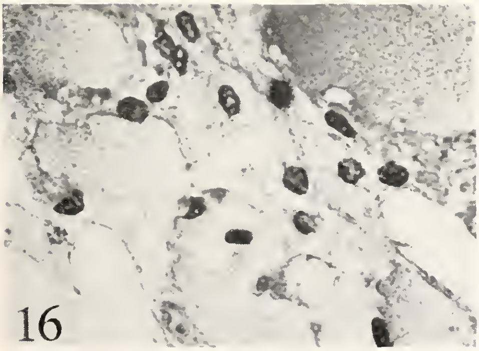
[5] All microphotographs were made using Bausch and Lomb microphotographic equipment. The photographs were taken on Eastman Super Panchro Press film, and were printed on Eastman Azo F-2, and Velour Black S-4 paper. Wratten filters G No. 15 and X-1 were used.


12


13


14


15


16

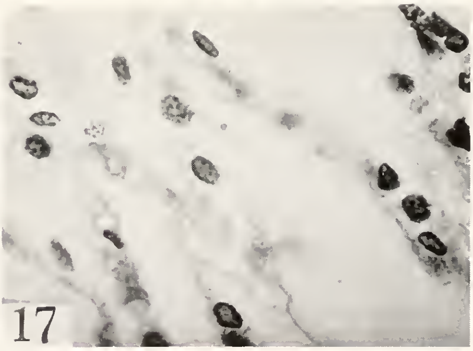

17

PLATE III

Hanström, 1939). In no stage of the adult is the sinus gland more than partially extruded beyond the level of the neurilemma. In all stages after the second, one finds a very large bundle of nerve fibers passing from the sinus gland toward the medulla terminalis. Some of these pass directly into the neuropile of the medulla terminalis and some ramify among the adjacent ganglion cells. In the first two adult stages the sinus gland is too closely appressed to the medulla terminalis for the presence of the nerve fibers to be readily determined. There is not, however, any evidence that there is a nerve fiber tract which extends directly to the brain as has been reported in Cambarus by Welsh (1941).

There are no obvious cell boundaries in the sinus gland. In fact there are so few nuclei to be found in any particular specimen's sinus gland that the individual cells which constitute the gland must be relatively very large. It is possible to detect canals which extend toward the blood sinus of the eye stalk; presumably these carry the secretory products to the blood stream. The secretions are in the form of large, more or less irregular, masses the amount of which varies very little regardless of the nearness or remoteness of the moulting period.

The secretions of the sinus gland give a basophilic reaction to the stains employed before, after and in the intermoult periods. In specimens fixed while in the process of moulting that portion of the sinus gland which is next to the neuropile of the adjacent optic ganglion gives an acidophilic reaction, whereas,

---

PLATE IV[6]

Microphotographs to show the sinus gland and X-organ in *Homarus americanus* and *Pinnotheres maculatus.* Figures 18, 19, 20, 22, 23 × 725, and figure 21 × 150; all are reduced about 25 percent. The material shown in figure 18 was fixed in Carnoy-Lebrun, that of figure 20 was fixed in Zenker-formol, and all others were fixed in Bouin-Duboscq-Brasil. The material shown in figure 22 was stained with Mallory's triple stain, and all others were stained with Foot's modification of the Masson trichrome stain.

FIGURE 18. *Homarus americanus.* A portion of the optic ganglion of an embryo fixed in the late egg stage and sectioned at five micra. The arrow indicates the characteristic secretory products of the X-organ which is surrounded by the cell layer of the optic ganglion. (Compare with Fig. 8.)

FIGURE 19. *Pinnotheres maculatus.* Section of the late egg stage embryo showing the position (arrow) of the X-organ. Sections were cut at four micra. (Compare with Fig. 1.)

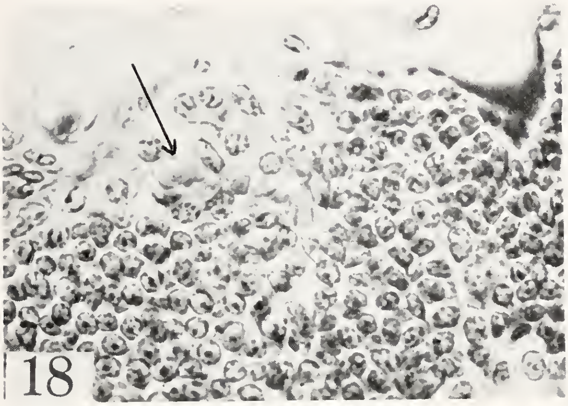
FIGURE 20. *Homarus americanus.* A portion of the eye stalk of a fourth stage embryo, sectioned at seven micra, showing the close association of the X-organ with the cells underlying the eye papilla. The bulge in the exoskeleton can be noted at the top of the photograph. Note that there are fewer nuclei in the X-organ, per unit area, than in the adjacent optic ganglion. (Compare with Fig. 10.)

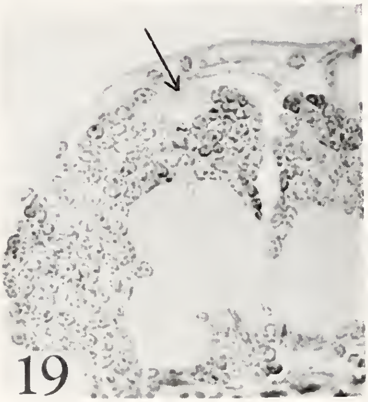
FIGURE 21. *Pinnotheres maculatus.* Section at eight micra of the eye stalk showing the general relationship of the various structures found therein. (Compare with Fig. 6.)

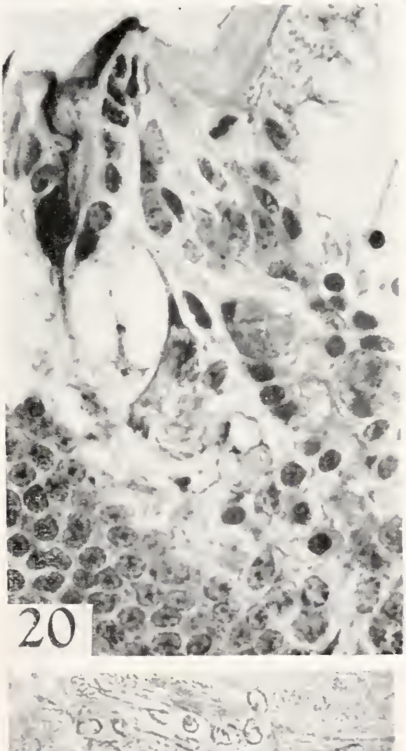
FIGURE 22. *Homarus americanus.* Section of the eye stalk of a third stage after hatching specimen (at seven micra) which shows the sinus gland lying just above the deeply staining muscle. Note that it stains much as the surrounding ganglion does and that the blood sinus is quite small.

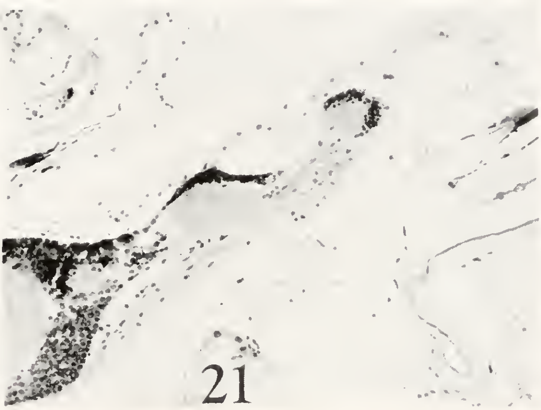
FIGURE 23. *Pinnotheres maculatus.* Section of the sinus gland shown in figure 21 enlarged to show its structure. This is the gland of a specimen that had been starved for forty-six days. Only one nucleus is to be found in this section, and what few secretory products are seen are stained brilliantly acidophilic. Note the indefiniteness to the connective tissue framework of the gland.

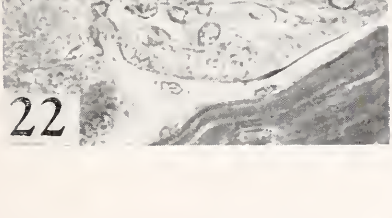
[6] All photographs, excepting that of figure 20, were taken using the same equipment and materials that were used for those of Plate III. The photograph for figure 20 was made on Eastman Ortho-X film using only the X-1 Wratten filter.


18


19


20


21


22

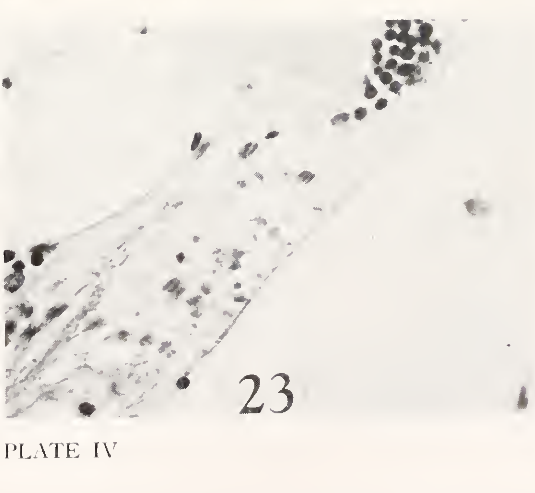

23

PLATE IV

the lateral portion, next to the blood sinus, gives a basophilic reaction. The acidophilic and basophilic portions blend together in the middle of the gland. Some specimens were starved for varying lengths of time. Those which had been starved for eight days exhibit both an acidophilic and basophilic reaction, but the two reactions are not regionally differentiated as is the case in specimens fixed while in the process of moulting. This reaction is found regardless of whether the specimens are fixed before, during or after the moulting period. In specimens starved for as long as 46 days (Figs. 21, 23) one finds only an acidophilic reaction regardless of the nearness or remoteness of the moulting period. Likewise, as the period of starvation is increased the amount of secretory material present in the gland is decreased although there is no evidence that the decrease due to starvation affects the frequency of moults in this particular animal.

B. *Homarus americanus*

The X-organ is found in the late egg stage (Figs. 8, 18); it is comparable in appearance to the X-organ in Pinnotheres, although there are definite structural differences in it. It is located in that part of the eye stalk that will become the median somewhat ventral side in the first stage after hatching. It is entirely surrounded by the cellular layer of the adjacent optic ganglion, but is separated from the ganglia cells by a thin connective tissue sheath. A definite bundle of nerve fibers extends from the X-organ to the medulla terminalis. The nuclei of the X-organ cells are histologically the same as those of the surrounding ganglia cells: the cytoplasm is more abundant than in the ganglia cells, and that which does not contain secretory products is clear and stains lightly. The secretory products show a series of concentric layers, when sectioned, comparable to those found in Pinnotheres; the nuclei are pushed to one side by the secretory products which nearly fill the entire cytoplasm. In all stages, under low power of the microscope, the X-organ has a similar appearance. This characteristic appearance has been described by Hanström (1939) as a "bunch of grapes." The distal portion of the X-organ extends to the median somewhat ventral portion of the hypodermis of the eye stalk. There is no evidence that there is any eye papilla formed at this time; in later stages the association of the X-organ and the eye papilla is evident. The X-organ exhibits no cyclic phenomena, cytologically, in the egg. The secretory products are always basophilic to the stains employed and vary very little in quantity.

In the first four stages after hatching (Figs. 10, 20) the X-organ increases greatly in size; this is due to the greatly increased number of cells in it and the increased amount of secretory products. Although there is a large increase in the number of cells found in the X-organ there is evidence of only an occasional mitosis after the animal has hatched. At its distal portion the X-organ comes into close contact with the exoskeleton which is bulged at this point. The cuticle of this particular region is extremely thin; this is the eye papilla (Figs. 10, 20). The eye papilla cells are found on the distal side of the X-organ between it and the ommatidia of the eye. There is no connection between the X-organ and the eye papilla as the X-organ is completely surrounded by a connective tissue sheath. No bipolar cells are found in the distal portion of the X-organ that are comparable to those described by Hanström (1937, 1939) for the adult of *Homarus* *americanus.* Cytologically there is no evidence of any cyclic phenomena in the X-organ during the first four stages after hatching; the secretory products are basophilic and the quantity is quite constant.

In the adult (Fig. 11) the X-organ no longer extends to the exoskeleton, but is found in the proximal half of the eye stalk. The basal portion of it is imbedded in the cellular layer of the distal part of the medulla terminalis. The distal portion extends well beyond the ganglionic cellular layer (to a point approximately level with the distal end of the medulla interna) into the blood sinus of the eye stalk. The X-organ occupies the same general position that it occupies in the earlier stages. In general structure it has become considerably more complex; it is now divided into a large number of units each of which is composed of from ten to twenty or more cells. Each of these units has a circular, whorled appearance. The nuclei are arranged around the periphery and the secretory products occupy the central area. A large bundle of nerve fibers passes around each of the units giving off nerve fibers to the individual cells. This arrangement gives the serial sections an appearance of being a series of whorls each of which originates from a common central stem of nerve fibers. The main bundle of nerve fibers passes between the various units and extends to the median side of the medulla terminalis. The nuclei have increased prodigiously in numbers, but still bear a marked resemblance to those of the cells of the optic ganglia. The cytoplasm of the X-organ cells is large and irregular in shape; it is filled for the most part with secretory products which have the characteristic concentric layers within them. Cells not possessing secretions have a clear lightly staining cytoplasm.

There is no evidence of cyclic phenomena associated with moulting as far as the X-organ is concerned. The basophilic reaction is found regardless of whether the eye stalk has been fixed a few days, 48 hours, six hours before, six hours, 48 hours, one, one and one-half, four or six months after moulting. Likewise, there is little change in the amount of secretory products that are evident in the X-organ of the eye stalks in the above series; the number of blue staining concretions is remarkably constant. In the case of the specimen that had not moulted for more than one year there were fewer secretory products present and more of the units contained vacuoles.

As far as can be determined the sinus gland is not formed sufficiently to be definitely recognized as such until the third stage after hatching (Figs. 9, 22). At this time it is a thin, lightly staining structure located on the dorso-lateral side of the eye stalk between the medulla interna and medulla externa. It is not very conspicuous as it does not give the typically brilliant acidophilic reaction to acid fuchsin that is found in the adult sinus gland. Structurally the sinus gland has the appearance of being a thickened portion of the neurilemma which invests the optic ganglia. The nuclei are few in number and stain precisely in the same manner as the nuclei of the neurilemma. The cell boundaries cannot be discerned; the cytoplasm seems to be confined to the connective tissue framework of the gland upon which the secretory materials are hung. The general tissue of the gland, regardless of what it is composed, stains very lightly with all the stains employed. There is a definite nerve fiber tract which extends from the sinus gland to the lateral distal border of the medulla terminalis. It is this fact that makes it possible to ascertain the presence of the sinus gland in the third stage after hatching. No such innervated structure has been found in the earlier stages.

In the fourth stage after hatching (Fig. 10) the eye stalk has increased more in thickness than in length. Consequently, the medulla interna is displaced; the sinus gland is found on the proximal portion of the medulla externa lateral to the medulla interna. This brings the sinus gland into closer proximity to the medulla terminalis. The sinus gland has increased in size with the resultant increase in the number of nuclei found in it, but the cytoplasm is still lacking the brilliant acidophilic reaction one might expect. No cell boundaries are visible; the nerve tract from the medulla terminalis is much more prominent than in the third stage after hatching.

In the adult (Fig. 11) the sinus gland occupies the same general position as in the early stages, but the eye stalk has become much more extended so that the medulla terminalis, interna and externa and lamina ganglionaris are strung out and occupy a much smaller portion of the inside of the eye stalk than they did in the early stages. As a result the sinus gland is found in the proximal half of the eye stalk on the opposite side from the X-organ. It is much more highly developed and extended than in the early stages. Situated alongside the medulla interna and extending to the proximal portions of the medulla externa it sends large finger-like processes out into the adjacent blood sinus. The nerve fiber tract extending from the sinus gland to the medulla terminalis is very large; after the protargol stain of Bodian (1937) one finds that the nerve fibers ramify among the fibers of the neuropile of the medulla terminalis and branch to all parts of the sinus gland. The framework of the gland is composed of connective tissue which stains precisely the same as the other connective tissue found in the eye stalk. There are no distinct cell boundaries observable in most preparations, but occasionally one is able to find an isolated cell which has a definite cell boundary surrounding a large irregular cytoplasmic mass. The nuclei have the same appearance as those of the early stages; they look more like connective tissue nuclei than nerve cell nuclei.

As has been pointed out above there is no striking staining reaction in the sinus gland of the third and fourth stages after hatching. In the adult, however, there are some interesting phenomena. In the series obtained for this research the following reactions are discernible: Specimens fixed several days and a few hours before moulting have the sinus gland filled with irregularily shaped secretory granules (Fig. 12) which, after Foot's modification of the Masson trichrome stain and other stains employing acid fuchsin and aniline blue, give a brilliant acidophilic reaction for the most part although there are a very few granules which react basophilically. Specimens fixed six hours, 48 hours, one and one and one-half months after moulting give three characteristic reactions. Some of the granules are brilliantly acidophilic, some are slightly acidophilic, and a number are decidedly basophilic (Fig. 13). In specimens fixed four and six months after moulting the amount of secretory material in the sinus gland is decidedly less than in those fixed during the summer months at or near the time of moulting (Fig. 14). In these cases the secretions are for the most part only slightly acidophilic with an occasional basophilic granule being found. In the specimen that had not moulted in over a year there was less secretory material in the sinus gland than was found in those (fixed in the summer months) which had moulted, but there was more than was found in those specimens fixed in the late fall and winter. The secretory material was brilliantly acidophilic, slightly acidophilic and basophilic. There was more basophilic material in this particular specimen than in any of the others. Examination of the exoskeleton showed that a new exoskeleton had been laid down underneath the old one which had not, for some reason, been shed.

C. *Cambarus virilis*

When the cyclic phenomena were found in the sinus gland of Homarus it was thought advisable to study the sinus gland of *Cambarus virilis* in which Dethier (1942) had previously reported a similar reaction. Accordingly, sections were made of the eye stalks of specimens fixed just before and just after moulting as well as of those fixed in late December. The sinus gland of specimens fixed just before moulting (Fig. 15) was filled with many irregularly-shaped granules which for the most part gave a brilliant acidophilic reaction, but there were occasional granules which were basophilic. In those specimens fixed after having completed moulting (Fig. 16) there was a sharp reduction in the number of secretion granules present; a few of these were brilliantly acidophilic, but most of them exhibited varying degrees of a basophilic reaction. In those specimens fixed late in December (Fig. 17) there were about the same number of granules as were found in the post-moult specimens, but the majority of these were acidophilic and only a few basophilic.

Discussion

Dethier (1942) in her account of the sinus gland in Cambarus states that she has been able to trace it from the first post-embryonic moult, and that it is apparently functional at that time.[7] This is not the case in the two species used for this investigation; in Homarus it has been impossible to ascertain definitely its presence until the third stage after hatching, and in Pinnotheres it could not be detected (with the techniques used) in the egg or first zoeal stages. Cytologically the evidence seems to indicate that the sinus gland in the third and fourth stages of Homarus is not a functional gland.

It has fairly well established that the color changes in Crustacea are controlled by hormones which originate in the eye stalk. As Kleinholz (1942) points out "the glandular tissue is probably the sinus gland, although the X-organ may also be concerned in this function." The apparent absence of the sinus gland in the early stages suggests that the X-organ may be functional in this capacity at this time, but the cytological evidence does not bear this out in Homarus and Pinnotheres. On the other hand, Cambarus has no X-organ which has the characteristic concretions of secretory material that are comparable to those found in Homarus and Pinnotheres. (Welsh, 1941, has found a mass of tissue on the dorso-lateral side of the medulla terminalis which he suggests may be the X-organ in *Cambarus bartoni.*)

Megušar (1912), Abramowitz and Abramowitz (1938, 1940), Brown and Cunningham (1939), Kleinholz and Bourquin (1941), and Smith (1940) have shown that the removal of both eye stalks from crustaceans hastens the onset of moulting. Smith showed quantitatively that the removal of both eye stalks shortened the intermoult period by slightly more than 30 per cent. This probably indicates that some structure in the eye stalk, possibly the sinus gland, produces a hormone which has an inhibiting effect upon moulting. Kyer (1942) gives good evidence that the sinus gland, when active, specifically inhibits moulting and gastrolith formation. Dethier (1942) in her account of Cambarus suggests that there is an acidophilic basophilic series which is related to the period of moulting. In the cases of Homarus and Cambarus the acidophilic reaction before moulting and the basophilic one after moulting seem to indicate cyclic changes in the sinus gland which are directly related to the moulting process. Further evidence of the activity of the sinus gland is exhibited by the reduction in the amount of secretory material in it; this is most striking in Cambarus, less evident in Homarus and scarcely detectable in Pinnotheres (this is probably due to the fact that Pinnotheres passes through several moults in fairly rapid succession).

---

[7] When the crayfish hatches it is a miniature adult with all appendages etc., and is comparable to a fifth or sixth stage of *Homarus americanus.*

The explanation of the basophilic and acidophilic reactions in Pinnotheres is more difficult on the basis of secretory activity. If one had only the normal animals to consider it might be possible to state that the activities of the sinus gland in this species passed through a reverse acid-base reaction which were a direct result of its activity. However, in as much as the sinus gland of the starved animals, and that in the ones in the process of moulting, both give acidophilic reactions it may be that the lack of food changes the pH- of the sinus gland from a normally basic range to an acid range. Since the animal does not feed during the period of ecdysis this may account in part for the acidophilic reaction of the sinus gland at this time.

Plankmann (1935) reported that various factors (starvation, etc.) may affect the rate of moulting. The Pinnotheres that were starved for varying periods of time were kept at a temperature comparable to that of their normal environment, on a dark background and in running sea water. There was no increase in the frequency or number of moults that occurred; it was the sinus gland that showed the affect of starvation and the X-organ appeared unchanged.

In the case of retinal pigment migration Parker (1897) could find no nerve fibers supplying the distal pigment cells in Palaemonetes. This observation started the controversy of the interrelationship of the eyes and subsequently many investigations have been made upon this subject. It has been shown in a generally satisfactory manner that the sinus gland produces a retinal pigment hormone (cf. Welsh, 1941). The question is raised as to the mechanism involved in the early stages where there is no obvious sinus gland to be found. If the sinus gland is the sole controlling factor it must be assumed that the early stages are incapable of retinal pigment migration.

Further studies are necessary to give satisfactory answers to the following points which have not been completely answered in the present study:

1. From precisely what pre-existing tissue is the sinus gland formed?
2. Is the sinus gland a syncytium?
3. Is the sinus gland noncellular and merely a storage space or are there cells which periodically fill with secretory products and break down (e.g. is secretion holocrine?)?

SUMMARY

1. The histogenesis of the sinus gland and X-organ have been studied and described for the egg, first zoea and adult stages of *Pinnotheres maculatus*.

2. The sinus gland is not found in the egg or first zoea, but it is found in all the adult stages of Pinnotheres.

3. The X-organ is found in the egg and other stages of Pinnotheres.

4. The histogenesis of the sinus gland and X-organ have been studied and described for the egg, first four stages after hatching and the adult of *Homarus americanus*.

5. The sinus gland is not found as a definitely discernible structure in Homarus until the third stage after hatching.

6. The X-organ is found in all stages of Homarus that have been studied.

7. Evidence is presented for the existence of cyclic secretion phenomena in the sinus gland of all species studied.

8. There is no evidence of the existence of cyclic secretion phenomena in the X-organ in any of the species investigated.

Acknowledgments

This research has been carried out with the aid of a fellowship granted by the Woods Hole Oceanographic Institution. I am indebted to Dr. John H. Welsh, at whose suggestion this problem has been carried out, for specimens of *Cambarus virilis* and the eye stalks of certain stages of the adult *Homarus americanus*, and for stimulating discussions of the problem. I am also indebted to Dr. Charles J. Fish of the Department of Zoology, Rhode Island State College, and the Wickford Hatcheries, Wickford, Rhode Island, for the eggs, the first four stages after hatching and the adult eye stalks (before, during and after moulting) of *Homarus americanus*. I wish to thank Dr. Gustavus H. Klinck, of the Samaritan Hospital, Troy, New York, for the use of the microphotographic apparatus.

LITERATURE CITED

ABRAMOWITZ, A. A., AND R. H. ABRAMOWITZ, 1938. On the specificity and related properties of the crustacean chromatophorotropic hormone. *Biol. Bull.*, **74**: 278–296.

ABRAMOWITZ, R. K., AND A. A. ABRAMOWITZ, 1940. Moulting, growth, and survival after eye stalk removal in Uca pugilator. *Biol. Bull.*, **78**: 179–188.

ATKINS, D., 1926. The moulting stages of the pea crab Pinnotheres pisum. *Jour. Marine Biol. Assoc.*, **14**: 475–493.

BODIAN, D., 1937. The staining of paraffin sections of nervous tissue with activated protargol. *Anat. Rec.*, 69: 153–162.

BROWN, F. A., AND O. CUNNINGHAM, 1939. Influence of the sinus gland of crustaceans on normal viability and ecdysis. *Biol. Bull.*, **77**: 104–114.

DETHIER, F., 1942. Cytological evidences for function in the sinus gland of the crayfish. Thesis, Harvard University. Unpublished.

FOOT, N. C., 1933. The Masson trichrome staining methods in routine laboratory use. *Stain Tech.*, **8**: 101–110.

HANSTRÖM, B., 1933. Neue Untersuchungen über Sinnesorgane und Nervensystem der Crustaceen. II. *Zool. Jb.* (*Abt. Anat.*), **56**: 367–520.

HANSTRÖM, B., 1934a. Neue Untersuchungen über Sinnesorgane und Nervensystem der Crustaceen. III. *Zool. Jb.* (*Abt. Anat.*), **58**: 101–144.

HANSTRÖM, B., 1934b. Über das Organ-X, eine inkretorische Gehirndrüse der Crustaceen. *Psychiat. Neurol. Bl. Amst.*, No. 3 en 4: 1–14.

Hanström, B., 1937. Die Sinusdrüse und der hormonal bedingte Farbwechsel der Crustaceen. *K. svenska. VetensAkad. Handl.* III., **16**: 1–99.

Hanström, B., 1939. *Hormones in invertebrates.* Oxford.

Kleinholz, L. H., 1942. Hormones in crustacea. *Biol. Rev.*, **17**: 91–119.

Kleinholz, L. H., and E. Bourquin, 1941. Effects of eye-stalk removal on decapod crustaceans. *Proc. Nat. Acad. Sci.*, **27**: 145–149.

Kyer, D. L., 1942. The influence of the sinus glands on gastrolith formation in the crayfish. *Biol. Bull.*, **82**: 68–78.

Megušar, F., 1912. Experimente über den Farbwechsel der Crustaceen. *Arch. Entw. Mech. Org.*, **33**: 462–665.

Parker, G. H., 1897. Photochemical changes in the retinal pigment cells of Palaemonetes, and their relation to the central nervous system. *Bull. Mus. Comp. Zool.*, **30**: 275–300.

Plankmann, H., 1935. Beitrage zur Physiologie der Garneelenhautung. *Schr. Naturw. Ver. Schl.-Holst.*, **21**: 195–216.

Scharrer, B., 1941. Endocrines in invertebrates. *Physiol. Rev.*, **21**: 383–409.

Sjögren, S., 1934. Die Blutdrüse und ihre Ausbildung bei den Dekapoden. *Zool. Jb.* (*Abt. Anat.*), **58**: 145–170.

Smith, R. I., 1940. Studies on the effect of eyestalk removal upon young crayfish (Cambarus clarkii, Girard). *Biol. Bull.*, **79**: 145–52.

Ståhl, F., 1938. Über das Vorkommen vom inkretorischen Organen und Farbwechselhormonen im Kopf einiger Crustaceen. *K. fysiogr. Sallsk. Handl. Lund*, **N. F. 49**: 1–20.

Welsh, J. H., 1941. The sinus gland and 24-hour cycles of retinal pigment migration in the crayfish. *Jour. Exp. Zool.*, **86**: 35–49.

NEURULATION IN MECHANICALLY AND CHEMICALLY INHIBITED AMBLYSTOMA

AGNES SANXAY BURT[1]

(*Department of Zoology, The University of Chicago*)

INTRODUCTION

Although the dependence of the medullary plate upon the chorda-mesoderm has attracted considerable attention from embryologists, the mechanism by which the plate becomes a neural tube has not been demonstrated.

In amphibians, it has been claimed that pressure exerted by ectoderm and mesoderm (Giersberg, 1924) or by the liquid confined between those two germ layers (Ruffini, 1925) is an active factor in neurulation. However, Lehmann (1926) and Boerema (1929), using different experimental approaches, have demonstrated that neurulation in these forms is an autonomous process within the medullary plate. In echinoderms (Moore and Burt, 1939; Moore, 1941) gastrular invagination, which in many respects resembles neurulation, has likewise been shown to be independent of ectodermal pressure.

Mitosis accompanied by a differential increase in cell volume has also been thought to be a factor in neurulation. Although little or no mitotic activity during this process was found by Glaser (1914) in *Cryptobranchus alleghenìensis* or by Ruffini (1925) in Triton, the latter worker believes mitosis to be a contributing factor to neurulation in Rana. Derrick (1937) reports that the high mitotic rate in the sides of the chick medullary plate as compared with the floor may aid neurulation in that form. In this animal it has also been found that after the neural tube has closed, incidence of mitosis is higher in the evaginating optic vesicles than it is in other regions of the brain (Frank, 1925). Hutchinson (1940), on the other hand, finds that the elongation of the neural tube which occurs soon after its closure in Amblystoma is not due to cell proliferation.

The hypothesis of Glaser (1914) that neurulation in Cryptobranchus may be caused by differential water absorption in the medullary plate cells has not been supported by the data of Brown, Hamburger, and Schmitt (1941) on Amblystoma. They find no appreciable increase in the water content of the plate during the critical period as determined by density measurements. Hobson (1941), however, was able to produce unfolding of partially closed chick neural tubes by dehydrating them in hypertonic media.

Ruffini (1925) reports that neurulation is aided by autonomous, amoeboid motion of the medullary plate cells. Boerema (1929) concludes that autonomous changes in cell shape are the responsible mechanism. It is well established (Goerttler, 1925; Vogt, 1929; Manchot, 1929, and many others) that extensive cell movements take place within the neural ectoderm which result in the elongation of the structure, but it is not known to what extent these movements are correlated with the formation of the neural tube.

---

[1] This investigation was carried out under the direction of Dr. Paul Weiss. It forms a part of a thesis on "Chemical Factors in Nerve Development" presented in partial fulfillment of the requirements for the Ph.D. degree. It has been supported by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

It was the purpose of the work reported here to compare the cellular changes taking place in normal embryos during neurulation with those in embryos in which neurulation had been inhibited by various means in an attempt to find some clue to the factors responsible.

MATERIALS AND METHODS

Several clutches of eggs of *Amblystoma maculatum* (Shaw) and of *Amblystoma tigrinum* (Green) were used, some of which were obtained near Chicago and some of which were shipped from Pennsylvania. The eggs were reared at room temperature unless otherwise noted, and care was taken that environmental conditions should be the same for experimentals and controls in a given series. Stage numbers of all specimens refer to Harrison's tables (1918, unpublished).

Most of the embryos were fixed in modified Formol-Zenker, double embedded in celloidin and paraffin, and sectioned at 6 micra. Some specimens were stained with Ehrlich's hematoxylin and mucicarmin for the study of cell shape, nuclei, and pigment granules; others were stained with neutral gentian violet to differentiate yolk and secretion granules. A few embryos were fixed in picric alcohol and stained with Best's carmine for the determination of glycogen.

MECHANICAL INHIBITION OF NEURULATION

Firstly, mechanical inhibition of neurulation was accomplished as follows: The medullary plates plus underlying mesoderm were excised from each of two *Amblystoma maculatum* embryos in Harrison's Stage 12 and explanted into Holtfreter's solution. One plate was then placed on top of the other and the two pieces of tissue weighed down with splinters of cover glass in such a manner that the plates could not fold up to form a tube. In some cases the plates were oriented so that the ectoderm of one was in contact with the mesoderm of the other; in other cases ectoderm was in contact with ectoderm. Six double explants of this type were studied. A number of intact Amblystoma eggs from the same clutch from which the membranes had been removed were reared in Holtfreter's solution, and 10 explanted medullary plates were allowed to develop freely in the same medium as controls.

The unoperated eggs developed normally except that, in some cases, the hypertonic medium caused a slight retardation of the head region. By the time the normal controls had reached Stage 28, the free explants showed distinct signs of neurulation. When the normal controls were in Stage 31 (Plate I, Fig. 1), the free explants had prominent neural folds which in some cases had nearly closed to form a tube (Plate I, Fig. 2). At the same time in the weighted explants, the medullary cells had elongated and become columnar as in early stages of normal neurulation, but the flask shape characteristic of later stages was never assumed and a tube was not formed (Plate I, Fig. 3).

There was no apparent difference in cell shape or intracellular organization between weighted explants whose ectoderm was in contact with ectoderm and those whose ectoderm was in contact with mesoderm. Thus it would seem that

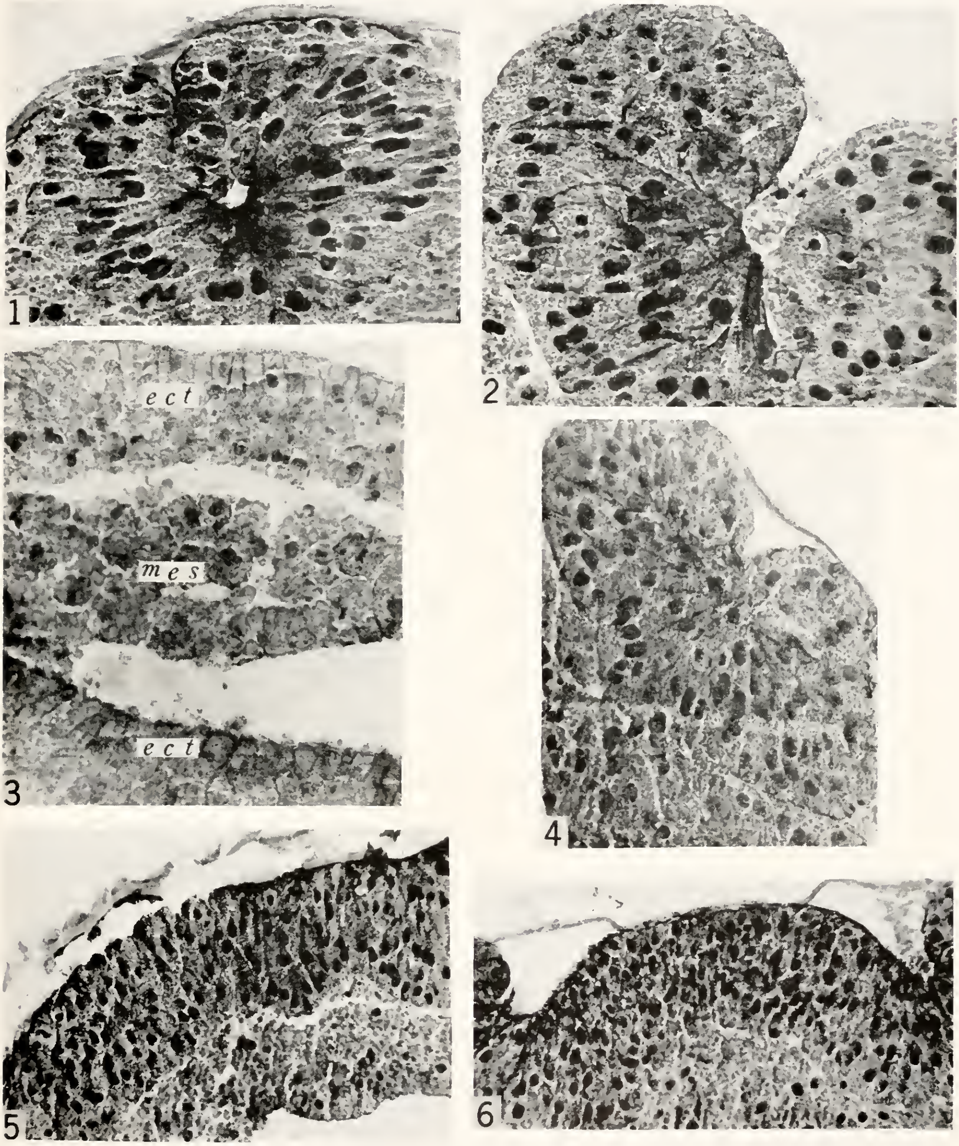


PLATE I

Figure 1. Neural tube of normal *A. maculatum* embryo. 250 ×.

Figure 2. Medullary plate from embryo of same chronological age as Figure 1 explanted into Holtfreter's solution. 250 ×.

Figure 3. Double explant, same age as Figure 1. ect. = neural ectoderm. mes. = mesoderm. 250 ×.

Figure 4. Normal *A. tigrinum*, Stage 18. 250 ×.

Figure 5. Ringer-treated embryo, same chronological age as Figure 4. 160 ×.

Figure 6. LiCl-treated embryo, same chronological age as Figure 4. 160 ×.

by Stage 12 the dorso-ventral polarity of the neural plate has already been established. In both the weighted and free explants the cells were rounder and shorter than those in the controls, the nuclei were round as compared to the oval ones in the normal animals, and there was a heavier deposit of pigment granules around the distal edges of the medullary cells. No other significant differences were noted.

From these data it was concluded that, while pressure at right angles to the plane of the medullary plate can inhibit closure of the neural folds, it does not suppress the initial cell elongation which accompanies that closure.

Chemical Inhibition of Neurulation

Next the developing eggs were subjected to the action of lithium chloride and of hypertonic salt solutions which, in the proper concentrations, will produce delayed closing of the neural tube or permanent *spina bifida*. Three series of experiments were carried out.

Table I

Comparison of development of normal, LiCl- *and Ringer-treated Amblystoma. Figures refer to Harrison's Stages*

	Normal controls	M/10 LiCl	Mammalian Ringer's	Remarks
Series 1				
A. tigrinum	19	15	16	
20° C.	29–30	16	18–19	
	34–35	18–19	20–21	neural tube still open in head region
Series 2				
A. maculatum	16–18	13	12	
12° C.	19–20	15	12	
	22–23	18–19	—	died about 140 hours after immersion in salt solution

The first series consisted of three groups of 33 *Amblystoma tigrinum* eggs which at the inception of the experiment were in Stage 13. The first group were reared in well water to serve as normal controls. The second group were reared in M/10 LiCl solution, the third in mammalian Ringer's. A second series consisted of three groups of 17 *A. maculatum* eggs which at the beginning of the experiment were in Stage 12 b. As with the *tigrinum* eggs, one group was reared in well water, one in M/10 LiCl, and one in mammalian Ringer's. However, the *maculatum* eggs, instead of being kept at room temperature, were placed on a water table with a practically constant temperature of 12° C.

The nervous systems of the treated animals in both series diverged considerably from the mean of normal development. In general, the head region was more retarded than the spinal cord. The approximate degree of maturity attained by the experimentals in comparison with the controls is shown in Table I. In staging the treated animals, external appearance was the criterion used.

It should be noted that the difference between the normal and lithium-treated embryos is greater at 20° C. than at 12° C. (this confirms the work of Hall (1942) on *Rana pipiens*) but that low temperatures apparently augment the effect of Ringer's solution.

The third series consisted of three groups of 17 *A. tigrinum* eggs which were in Stage 11 b—12 a at the beginning of the experiment. One group served as normal controls, one group was immersed in M/20 LiCl for 24 hours, after which development was allowed to continue in well water, and the third group was similarly treated with M/20 NaCl. NaCl treatment had no perceptible effect on the rate or type of development, while the equimolar LiCl solution retarded the embryos considerably. This series of eggs was fixed in picric alcohol for a rough determination of glycogen content.

Effects of chemical inhibition on mitotic rate

The effects of chemical inhibition were best seen in the first series of eggs as the Ringer-treated eggs did not develop at all in the second series. One-third of the embryos were fixed and sectioned when the normal controls were in Stage 18 (at which time the normal germs had open medullary plates with well raised neural folds), one-third when the controls were in Stage 30, and the remainder when the controls were in Stage 35, by which time the lithium embryos were in approximately the same stage of development as the controls at Stage 18 as far as external appearance was concerned, and the Ringer-treated germs were slightly more mature. The effect of this inhibition on the mitotic rate in the medullary plate is summarized in Table II.

TABLE II

Mitotic rate in the medullary plate of A. tigrinum. Stage numbers not in parenthesis refer to normal controls; those in parenthesis refer to inhibited animals of the same chronological age as the normal controls

	Stage	Cells counted	Mitoses seen	Mitotic index
Normal controls	18 30 35	1461 776 1037	39 29 42	2.67% 3.73% 4.05%
Lithium chloride-treated	18(15) 30(16) 35(18)	1970 1109 693	19 12 3	0.96% 1.08% 0.43%
Ringer's treated	18(16) 30(18) 35(20)	2276 760 1074	28 18 30	1.23% 2.37% 2.79%

From this it is apparent first, that there is mitosis in the neural plate of *A. tigrinum* during neurulation; secondly, that the mitotic rate rises in the normal animal after the neural tube is closed; thirdly, that Ringer's solution depresses the mitotic rate in comparison with normal embryos of the same chronological age, but that the mitotic rate in Ringer-treated animals is comparable to that in normals of the same stage of development, and fourthly, that LiCl causes both a relative and an absolute decrease in the mitotic rate of the neural tube.

Effects of chemical inhibition on cell shape

The effects of inhibition on the cellular morphology of the neural tube were extreme. When the normal controls were in Stage 18 (Plate I, Fig. 4), the Ringer-treated animals showed a slight evagination of the floor of the medullary plate (Plate I, Fig. 5), and lithium-treated embryos a very marked evagination (Plate I, Fig. 6).

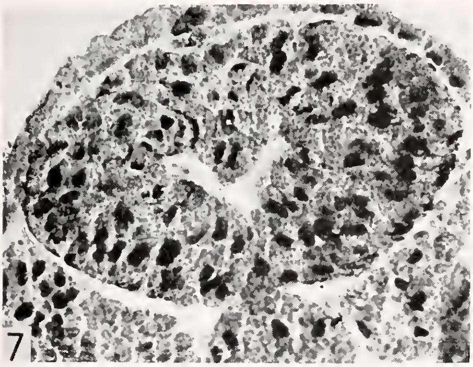
By the time the normal controls were in Stage 30 (Plate II, Fig. 7), ectoderm had begun to grow over the edges of the plate in the Ringer-treated germs and a slight invagination of the plate was present (Plate II, Fig. 8). When the controls were in Stage 35 (Plate II, Fig. 10) and the Ringer-treated embryos in what corresponded to Stage 20 in the normal animals, the invagination was fairly deep in the treated germs and the edges of the plate were raised, although they were not bent over as normal neural folds are at that time (Plate II, Fig. 11).

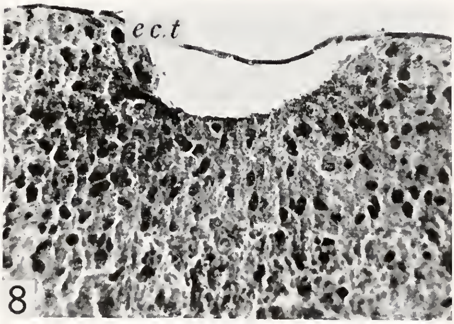
In the LiCl-treated germs, on the other hand, when the controls were in Stage 30, a flat plate was present (Plate II, Fig. 9). When the controls had reached Stage 35 and the lithium-treated animals were in Stage 18 as far as external appearance was concerned, the neural plate was still flat, but a few flask-shaped cells had appeared at the edges as in the first stages of normal neurulation. Many of the medullary plate cells in these embryos, particularly in the head region, became round and sloughed off into the space above the plate (Plate II, Fig. 12). Child (1941) reports a similar dissociation of the endodermal plate in the starfish, Pateria, when exposed to the action of lithium chloride.

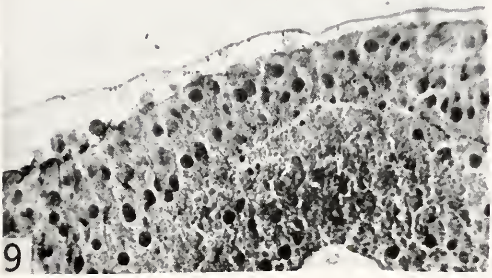
The changes in shape occurring in both the normal and treated embryos naturally correspond to the changes in the shape of the plate as a whole. These changes may be summarized by saying that both Ringer and LiCl treatment produce, first, a more or less evaginated medullary plate and then a flat or slightly invaginated plate which may, according to the concentration of the chemicals used, proceed to form a tube in places or to be overgrown by ectoderm, and that no traces of a neural keel as described by Baker (1927) were seen in the treated embryos in the series studied.

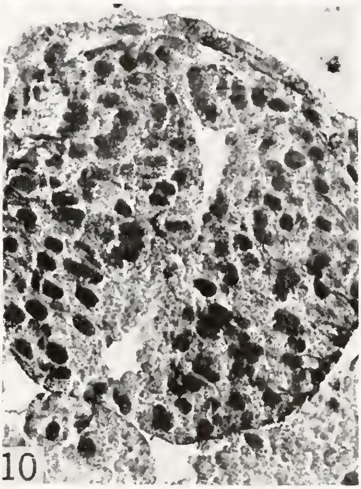
Effects of chemical inhibition on nuclear size

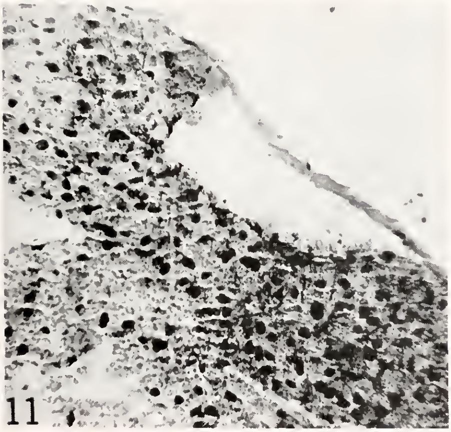
Much importance has been attached to changes in cell and nuclear size during neurulation since Glaser (1914) found that, in Cryptobranchus, the volume of the neural plate increased during the course of neurulation and believed that this indicated an increasing water content of the neural plate. He also inferred that increased hydration occurs during gastrulation in echinoderms because of reported increases in nuclear size during that process. As Brown, Hamburger, and Schmitt (1941) found no indications of increased hydration in density measurements on Amblystoma, an effort was made to throw more light on the problem by measuring the nuclear axes of 100 medullary plate cells in both normals and experimentals in each of three stages. As these nuclei are not perfect spheres and as their orientation varies somewhat within the plate, these measurements cannot be used to calculate nuclear volume. However, any large changes in nuclear volume should be revealed by this method. Indices of nuclear area and shape were also calculated. These data are summarized in Table III.











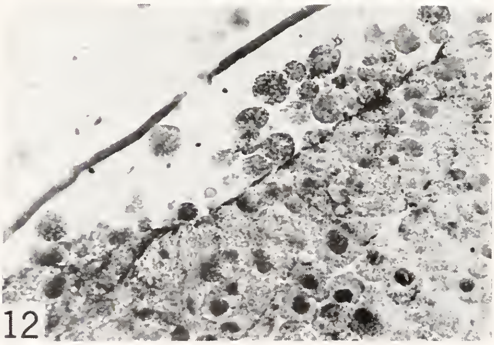


PLATE II

FIGURE 7. Normal *A. tigrinum* embryo, Stage 30. 250 ×.

FIGURE 8. Ringer-treated embryo, same chronological age as Figure 7. ect. = ectoderm growing over medullary plate. 160 ×.

FIGURE 9. LiCl-treated embryo, same chronological age as Figure 7. 160 ×.

FIGURE 10. Normal *A. tigrinum* embryo, Stage 35. 250 ×.

FIGURE 11. Ringer-treated embryo, same chronological age as Figure 10. 160 ×.

FIGURE 12. LiCl-treated embryo, same chronological age as Figure 10. Note round cells sloughed off from plate. 250 ×.

No statistically significant differences in nuclear axes, area, or shape were revealed by this analysis between normal and treated nuclei because of the large standard deviations involved. However, it should be noted that the index of shape (A/B) increased consistently in the normal germs whereas it remained practically constant or decreased slightly in the chemically treated germs. This lack of nuclear elongation seems to be correlated with the failure of cell elongation which was also observed in these cases. While no conclusions can be drawn from these findings as to cellular hydration, there is no change of nuclear size during folding of the neural plate.

Effects of chemical inhibition on cellular inclusions

A. *Yolk granules.* In normal *A. tigrinum* and *A. maculatum* embryos, yolk begins to be utilized in the neural tube, beginning in the head region, about Stage 18. By Stage 35, yolk has practically disappeared from the brain, and only a sparse scattering of granules remains around the lumen of the spinal cord. Bragg (1939), who has studied the utilization of yolk in a number of other amphibian genera, reports that in his animals it did not begin until after the closure of the neural tube.

TABLE III

Greatest nuclear length (A) *and diameter* (B) *of 100 medullary plate cells. Stage numbers not in parenthesis refer to normal controls; those in parenthesis refer to treated animals. All measurements are in ocular micrometer units*

	Stage	Nuclear measurements				Indices			
		Length (A)		Diameter (B)		Area (AB)		Shape (A/B)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Normal controls	18	15.00	2.22	8.84	1.58	129.4	34.8	1.72	0.35
	30	14.60	2.76	8.42	1.56	119.2	30.6	1.80	0.51
	35	16.80	2.83	8.86	1.52	146.6	36.8	1.93	0.48
Ringer-treated	18(16)	13.55	2.41	8.66	1.56	115.9	33.4	1.59	0.38
	30(18)	14.33	2.24	9.03	1.71	126.7	38.8	1.58	0.36
	35(20)	13.27	2.18	9.42	1.56	121.7	32.2	1.42	0.32
LiCl-treated	18(15)	14.79	2.21	9.51	1.46	139.7	34.4	1.45	0.27
	30(16)	14.63	2.22	10.79	1.56	157.3	38.0	1.26	0.25
	35(18)	13.96	2.03	10.02	1.17	138.7	32.0	1.38	0.20

Lithium chloride and Ringer's solution both seem to retard the disappearance of yolk as well as the closure of the neural tube, as the medullary plates of the treated animals were packed with yolk granules throughout the period of observation whereas, in the normal controls, the amount significantly diminished. It is doubtful if this is causally related to the process of neurulation, however, because (1) yolk disappears very late in this process and (2), as Morgan (1906) has shown, eggs of *Bufo variabilis* centrifuged so that all granules are thrown out of portions of the head in the resultant embryos will develop closed neural tubes.

B. *Glycogen.* In the *A. tigrinum* series, no perceptible change in the glycogen content of the nervous system was noted between Stage 18, at which time the neural tube is open over its full length, and Stage 30, when the entire tube is closed and morphogenesis of the brain is well under way. All the neural cells contained much glycogen, no significant differences being noted among the various regions of the nervous system.

Treatment with M/20 NaCl did not affect glycogen distribution (as was to be expected since no morphological changes were observed) nor did treatment with M/20 LiCl. Thus, although no histological method is exact enough to reveal very small changes in glycogen content, it would appear that in *A. tigrinum* neurulation is not accompanied by significant utilization of this material.

C. *Pigment granules.* Early in the normal process of neurulation in Amblystoma, as reported by Ruffini (1925) and Lehmann (1926) for other urodeles, there is a marked accumulation of pigment, especially in creases formed by the medullary folds. From the pigment layer at the distal ends of the cells, rows of pigment granules extend along the cell boundaries (see Plate I, Fig. 1). When neurulation is completed, there is a layer of pigment granules along both surfaces of the neural tube and many granules along the cell boundaries and within the cell bodies.

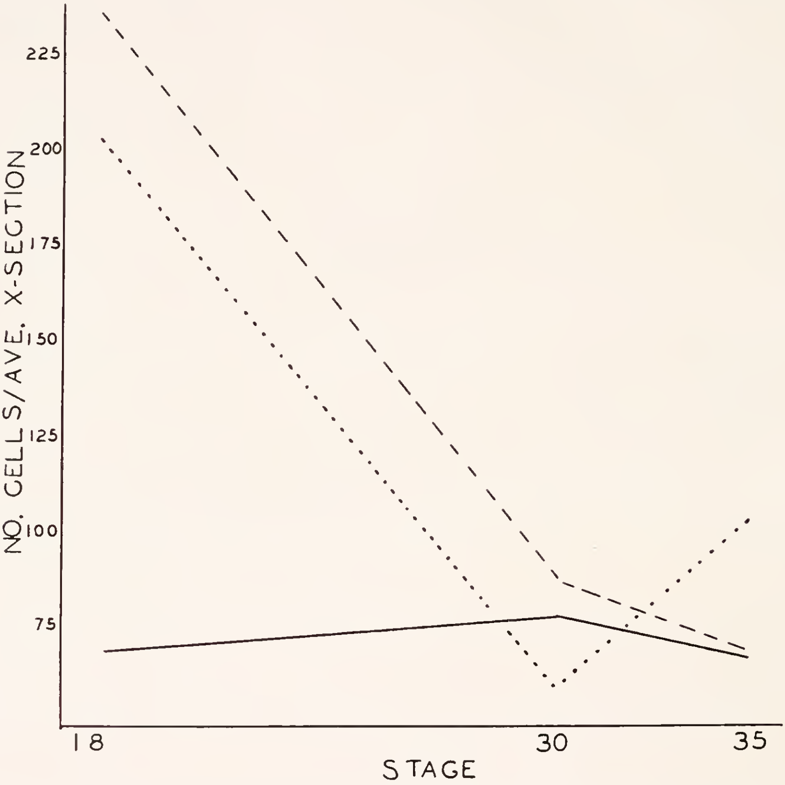
The chief difference noted in the treated animals was that the concentration of pigment near the outer surface of the plate cells occurred irregularly and only in those cells where shrinkage of the inner surface took place (see Plate I, Figs. 5 and 6; Plate II, Figs. 8 and 12). In the lithium-treated specimens, as soon as degeneration of the plate commenced, many granules escaped into the free space above the plate, and all of the sloughed-off cells were packed with pigment. These granules, like yolk, however, appear to be a passive factor in neurulation and are of value only as an indicator of the results of active processes which change cell shape.

D. *Secretion granules.* As shown by Studnička (1900) and Weiss (1934), secretion occurs in the embryonic ependyma. On the chance that secretion might be involved in the process of neurulation, normal and chemically inhibited *A. tigrinum*, *A. maculatum*, and *Rana pipiens* germs and normal chick embryos which had been stained with neutral gentian violet were examined for secretion granules. None were found, either in the normal embryos or in the treated amphibians. This does not necessarily indicate that unformed secretion does not occur; in fact, the presence of liquid within the lumen of the neural tube is evidence that secretion of some sort does take place very early in normal development. Because of the difficulty of demonstrating secretion antecedents, however, the problem requires study by more refined techniques.

Effects of chemical treatment on cellular movements

The most accurate method of following cell movements in embryonic development is by vital staining, a procedure not used in this investigation. However, some indication of those movements was obtained by counting the number of cells in the neural plate in every fifth section of the trunk region of embryos (exclusive of brain and tail) and averaging the results. It is very hard to obtain strictly comparable results by this method because, as Manchot (1929) has shown, in the normal development of urodele embryos, the anterior two-thirds of the neural plate becomes brain while the posterior one-third elongates to form the spinal cord. A rough comparison between the normal and chemically treated neural plates at various stages of development is presented in Graph 1.

As this graph was constructed from data on only nine animals, not too much significance can be attached to it. However, it would seem that in normal animals between Stages 18 and 35 mitosis and stretching of the neural plate keep pace with one another so that the average number of cells per cross section remains approximately constant and that, although LiCl and Ringer's solution do inhibit the stretching process just as they inhibit neurulation and mitosis, elongation of the neural plate continues under their influence.[2]



GRAPH 1. Average numbers of cells present in every fifth section of the medullary plate of normal and chemically treated *A. tigrinum* embryos of the same chronological age. Abscissae are stage numbers of normal controls. Solid line used for normal controls, dashes for lithium embryos, dots for Ringer germs.

---

[2] In Glaser's work on Cryptobranchus (1914), he used the average number of nuclei present per cross-section to test whether or not cell division was occurring. Because the number of nuclei remained approximately constant, as it does in normal Amblystoma during slightly later stages, he concluded that there was little or no mitosis during neurulation. It would be interesting to re-examine his material to see if elongation of the medullary plate played any role in that constancy.

Discussion

In discussing neurulation, it must be borne in mind that the folding of the neural plate is a very complex process involving not only changes in cell size and proportions, but physical and biochemical changes which have, as yet, been little studied. From the foregoing analysis, it can be concluded that certain factors are not involved in the more obvious phases of folding. Thus not only does neurulation occur, as has been previously reported by many workers, in the absence of normal mechanical pressures, but the characteristic preliminary cell elongation takes place when pressure is exerted at right angles to the usual direction of cell movement.

Neurulation seems to be independent of nuclear area, and if, as has been suggested, the latter be accepted as an index of cell hydration, also independent of hydration. For, although treatment with Ringer's solution and LiCl had no significant effect on nuclear area, it did inhibit folding of the medullary plate. On the other hand, nuclear elongation, which accompanies cell elongation in the normal plate, does not occur when folding is inhibited.

Judging by the data on average number of cells per section, the elongation of the medullary plate which normally takes place during and after neurulation is not necessarily correlated with the closure of the neural tube, because, although both LiCl and Ringer's solution do retard the stretching process somewhat, it continues even when neural folds do not form.

Mitosis seems to be either directly instrumental in neurulation or at least under control of the mechanism of neurulation. Thus in the LiCl-treated embryos, in which the mitotic rate fell to a very low value during the experiment, no folds appeared, while the elevation of the sides of the neural plate in both the normal and the Ringer-treated specimens was accompanied by active cell division.

Cell elongation and wedging are unavoidably correlated with embryonic folding, and, as Boerema (1929) and others have pointed out, such changes are theoretically quite sufficient to cause neurulation. As demonstrated by Brown, Hamburger, and Schmitt (1941) differential water absorption cannot account for such changes in Amblystoma. These workers and Schmitt (1941) independently have suggested that molecular interactions and desolvations in the cell surface may exert the forces necessary to cause cell elongation. Weiss (unpublished) has suggested further that the concentration of pigment granules which occurs in the normal folding plate indicates a contraction of the cell cortex at the free surface. Although Hobson (1941) has not succeeded in demonstrating any systematic changes in the ultrastructure of the chick neural plate during folding by polariscopic analysis, a more intensive investigation of such changes during neurulation appears to be the most promising method of approach to the problem.

An interesting point which emerges in a comparison of the LiCl and Ringer-treated germs is that the effects of the two agents on neurulation seem to be produced by different means. Thus lithium is less effective at low temperatures, while hypertonic salt solutions are more effective. Further, lithium salts inhibit neurulation at much lower concentrations than do those present in Ringer's solution. Hall (1942) has evidence that lithium is a toxic agent acting on the chordamesoderm rather than on the responding ectoderm. The fact that Ringer's solution is a more effective inhibitor at low than at high temperatures would suggest that it acts on the physical consistency of the embryo rather than on chemical processes, perhaps by stiffening the neural plate so that folding is impeded—a suggestion which Giersberg (1924) has previously offered to explain the action of sucrose and sodium acetate on neurulation.

Finally, it should be noted that the data presented here are by no means conclusive in themselves; they are offered merely in an effort to shed light on a few phases of a very complicated problem.

SUMMARY

1. Mechanical pressure exerted at right angles to the plane of explanted medullary plates has been found to suppress neurulation in *Amblystoma maculatum*, but not the preliminary cellular elongation which is normally involved in that process. This elongation takes place irrespective of whether the medullary plate is in contact with ectoderm or with mesoderm on the normally free surface.
2. In *Amblystoma tigrinum* and *A. maculatum* neurulation is accompanied by mitosis, the mitotic rate rising after the neural tube has closed. Treatment with mammalian Ringer's solution at room temperature decreases the mitotic rate to about the same degree as it inhibits normal development; treatment with M/10 LiCl decreases the mitotic rate both relatively and absolutely.
3. No statistically significant difference was found in average nuclear area between normal and treated medullary plates. In normal germs, the nuclei elongate during neurulation, whereas in the treated germs they did not.
4. Glycogen and yolk begin to disappear from the normal neural tube about Stage 18. Neurulation-inhibiting chemicals retard the utilization of these substances.
5. Pigment granules appear to be passive factors in neurulation indicative of contraction at free cell surfaces.
6. No evidence of formed secretion from the neural plate was found.
7. Although inhibiting chemicals decrease the rate of elongation of the medullary plate, stretching continues even when neural folds fail to form.
8. The inhibiting action of LiCl is less effective at low temperatures, that of Ringer's is augmented.
9. It is concluded that neurulation in Amblystoma is autonomous to the medullary plate and may be aided by mitotic activity; changes in nuclear area (which may be indicative of cell hydration), intracellular inclusions, and longitudinal cell movements are not instrumental in the process.

LITERATURE CITED

BAKER, R. C., 1927. The early development of the ventral part of the neural plate of Amblystoma. *Jour. Comp. Neur.*, **44**: 1–27.

BOEREMA, I., 1929. Die Dynamic des Medullarrohrschlusses. *Arch. f. Entwmech.*, **115**: 601–615.

BRAGG, A. N., 1939. Observations upon amphibian deutoplasm and its relation to embryonic and early larval development. *Biol. Bull.*, **77**: 268–284.

BROWN, M. G., V. HAMBURGER AND F. O. SCHMITT, 1941. Density studies on amphibian embryos with special reference to the mechanism of organizer action. *Jour. Exp. Zool.*, **88**: 353–372

CHILD, C. M., 1941. *Patterns and problems of development.* University of Chicago Press.

DERRICK, G. E., 1937. An analysis of the early development of the chick by means of the mitotic index. *Jour. Morph.*, **61**: 257–284.

FRANK, G. M., 1925. Über Gesetzmässigkeiten in der Mitosenverteilung in den Gehirnblasen in Zusammenhange mit Formbildungsprozessen. *Arch. f. Entwmech.*, **104**: 262–272.

Giersberg, H., 1924. Beiträge zur Entwicklungsphysiologie der Amphibien. II. Neurulation bei Rana und Triton. *Arch. f. Entwmech.*, **103**: 387–424.

Glaser, O. C., 1914. On the mechanism of the morphological differentiation in the nervous system. I. The transformation of a neural plate into a neural tube. *Anat. Rec.*, **8**: 525–551.

Goerttler, K., 1925. Die Formbildung der Medullaranlage bei Urodelen. *Arch. f. Entwmech.*, **106**: 503–541.

Hall, T. S., 1942. The mode of action of lithium salts in amphibian development. *Jour. Exp. Zool.*, **89**: 1–36.

Hobson, L. B., 1941. On the ultrastructure of the neural plate and tube of the early chick embryo, with notes on the effects of dehydration. *Jour. Exp. Zool.*, **88**: 107–134.

Hutchinson, C., 1940. A study of medullary plate formation in Amblystoma punctatum. *Anat. Rec.*, **78** (Suppl.): 56.

Lehmann, F. E., 1926. Entwicklungsstörungen in der Medullaranlage von Triton, erzeugt durch Unterlagerungsdefekte. *Arch. f. Entwmech.*, **108**: 243–282.

Manchot, E., 1929. Abgrenzung des Augenmaterials und anderer Teilbezirke in der Medullarplatte; die Teilbewegungen während der Auffaltung (Farbmarkierungsversuche an Keimen von Urodelen). *Arch. f. Entwmech.*, **116**: 689–708.

Moore, A. R., 1941. On the mechanics of gastrulation in Dendraster eccentricus. *Jour. Exp. Zool.*, **87**: 101–111.

Moore, A. R., and A. S. Burt, 1939. On the locus and nature of the forces causing gastrulation in the embryos of Dendraster eccentricus. *Jour. Exp. Zool.*, **82**: 159–171.

Morgan, T. H., 1906. The influence of a strong centrifugal force on the frog's egg. *Arch. f. Entwmech.*, **22**: 553–563.

Ruffini, A., 1925. Fisiogenia. La biodinamica dello sviluppo ed i fondamentali problemi morfologici dell'embriologia generale. F. Vallardi, Milan.

Schmitt, F. O., 1941. Some protein patterns in cells. *Growth*, **5** (Suppl.): 1–20.

Studnička, F. K., 1900. Untersuchungen über den Bau des Ependyms der nervösen Centralorgane. *Anat. Hefte*, **15**: 303–431.

Vogt, W., 1929. Gestaltungsanalyse am Amphibienkeim mit örtlicher Vitalfärbung. II. Teil. Gastrulation und Mesodermbildung bei Urodelen und Anuren. *Arch. f. Entwmech.*, **120**: 384–706.

Weiss, P., 1934. Secretory activity of the inner layer of the embryonic mid-brain of the chick. *Anat. Rec.*. **58**: 299–302.

ANALYSIS OF POPULATION DEVELOPMENT IN DAPHNIA AT DIFFERENT TEMPERATURES

DAVID M. PRATT

(From the Biological Laboratories, Harvard University, Cambridge)

INTRODUCTION

The purpose of this study was to analyze the development of Daphnia populations under controlled conditions in which temperature was the chief variant. It was proposed to investigate not only the effect of temperature upon the rate of increase, but also its influence upon subsequent changes in the numerical strength of the population. The original intention was to measure the effect of temperature by comparing the equilibrium values, i.e. asymptotes, attained by populations at different temperatures, and through further experiments to identify the processes by which temperature might act to bring about the observed differences. However, the type of population growth curve obtained precludes the comparison of asymptotes and necessitates a brief historical sketch of population studies in general and the curves developed from them, in addition to a review of previous research upon the effects of temperature.

Since the animal chosen for the investigation is a planktonic form, the analysis of the relation of temperature to the development of a population acquires additional interest from an old controversy. It has long been held that the polar regions support a more abundant, if a less diversified, fauna and flora than do the tropics. This contention has been stressed especially in connection with the latitudinal distribution of plankton, and a number of theories have been advanced relating temperature, directly or indirectly, to the density of planktonic populations. The relation of the present study to this problem and other possible applications will be dealt with in the discussion.

HISTORICAL BACKGROUND

The logistic equation and its sigmoid curve, rediscovered by Pearl and Reed (1920), have been applied to the study of human populations (Pearl, 1925) and experimental populations of a variety of organisms, including yeast cells (Clark, 1922; Richards, 1928), diatoms (Ketchum and Redfield, 1938), infusorians (Robertson, 1921, 1923), and flour beetles (Chapman, 1928; Holdaway, 1932). Indeed no population study under controlled environmental conditions has demonstrated any other type of population growth curve. It should be noted, however, that the interest of investigators of experimental population development has been focussed almost exclusively on the early parts of the growth curve, with very little regard for the important part of the history which follows the initial period of increase. Although the definition of a logistic curve requires an upper asymptote, some workers have followed the development of their experimental populations only to the point where they first approach an apparent maximum size, and in presenting their results have termed their curves logistic. While it is true that in several studies (e.g. Pearl with Drosophila, 1925; Gause with yeast, 1932; Chapman with Tribolium, 1928) the population has maintained an upper asymptote for a period long compared to the "growth" period, it does not follow that populations of other species or under different circumstances would yield similar results. The tacit assumption that an asymptote can be calculated from the maximum size reached by a population, without experimental evidence that a state of relative equilibrium has been attained, is entirely gratuitous. It may well be that the failure to demonstrate, hitherto, a type of population growth curve that is not logistic after the initial period of increase, has been due in part to this fallacious assumption.

The rather extensive literature on population studies yields but a meager amount of information concerning the effects of temperature. The yeast *Saccharomyces cerevisiae* has been the subject of two investigations involving temperature. Richards (1928a) found that the rate of multiplication increases with temperature between 4° and 30° C.; above this range it decreases. In a more thorough analysis of the effects of temperature, Gause (1932) followed the development of yeast populations to their asymptotes and discovered that in a temperature range of 5.7° to 41.0° C. the relation between the size of the asymptotic population and the temperature can be expressed by a bell-shaped curve with the mode at about 24° C. In the same paper Gause reported that populations of Drosophila held at 29° C. attain an asymptote of 310, whereas at 30° the asymptote is only 146.

Terao and Tanaka (1928, 1928a, 1928b, 1930) attempted the study of the influence of temperature on population development in *Moina macrocopa*, but followed their population growth curves only to apparent maxima, and based their conclusions on the calculated values of undemonstrated asymptotes.

Materials and Methods

Daphnia magna would appear to be ideal material for population studies because of its size, high reproductive capacity, and parthenogenesis, which makes it easy to obtain genetically identical material. Daphnia is less easily provoked to the production of males and sexual females than other cladoceran genera (e.g. Moina), so that in a crowded population a very high percentage of the individuals are potential producers, and there is no problem of a proper balance of sexes. In these experiments the sex ratio was noted at irregular intervals, and at no time did the males constitute more than 10 per cent of the population. Since cladocerans pass their early stages in the maternal brood pouch whence they are released in active state, there are no stages (such as eggs) so small as to require special precautions against loss during transfer of the population to fresh medium.

The populations developed in 50 cc. of filtered pond water from the Middlesex Fells, in open, widemouthed glass bottles whose water-surface area was 10.9 cm.^{2} The seeding of each bottle was two animals (parthenogenetic females) that had been released from the brood pouch within 24 hours. Each population was counted every two days, at which time the dead were removed, their number noted, and the water changed. This was done by pouring the contents of each bottle into a fingerbowl, whence the animals were transferred with a pipette to a second fingerbowl containing about 50 cc. of fresh pond water at the same temperature, and thence into a clean bottle which was finally filled up to the 50 cc. mark with fresh pond water. By this rinsing process the small amount of used water carried over in the pipette was greatly diluted. Thus "conditioning" of the medium by the metabolic activities of the animals was never allowed to proceed for more than two days.

The only food used was *Chlorella pyrenoidosa*, a unicellular green alga that will grow in a thick suspension when properly cultured (in Detmer's Solution, exposed to neon light, with carbon dioxide bubbling through the medium). It was found necessary to culture the Chlorella under sterile conditions to prevent the development of a concentrated bacterial flora in the culture flasks. In previous experiments contamination of the Daphnia medium from this source had occasionally been sufficiently severe to injure the animals. The quantity of food given each population was not measured by any absolute standard. In each case it was roughly calculated, by previous experience alone, to exceed the requirements of the particular population. This method proved entirely satisfactory, for the medium always had a distinct greenish tinge. It was also demonstrated by simple experiments that when the concentration of the Chlorella was half as great or several times as great as the concentration that normally would have been used under the given conditions, the longevity and reproductive rate of the animals were not appreciably affected. Therefore neither a lack nor an excess of food was ever a limiting factor in the growth of the populations.

The temperatures chosen for comparison were 12°, 18° and 25° C., covering a considerable portion of the range (8° to 28° C.) demonstrated suitable for the life and reproduction of *Daphnia magna* (MacArthur and Baillie, 1929). At 12°, however, populations persisted for only a few weeks of faltering growth and rapidly dwindled to extinction. Under the ecological conditions that obtained, apparently the metabolic rate was not high enough to insure the reproductive and survival rates requisite for population growth and maintenance. In consequence the lowest temperature was abandoned and the work was limited to two temperatures, 18° and 25° C. The populations were maintained at these temperatures (plus or minus 1° C.) by keeping them in incubators in a cold-room.

The culture bottles were placed in daylight from a north window. However, all the populations received approximately the same amount of light. Aside from this, no attempt was made to control light conditions, which varied from day to day and from season to season.

The only environmental agencies that suggest themselves as possible limiting factors in the growth of populations of such an animal as Daphnia are: 1. exhaustion of the food supply and 2. conditioning of the medium by the accumulation of metabolites and/or depletion of the dissolved oxygen. Since the former was never operative in these experiments, any limitation in the increase in numbers must have been the expression of some form of conditioning of the medium, although that process was never continuously sustained for more than two days. In an attempt to ascertain the nature of this conditioning, the concentrations of hydrogen ion, dissolved oxygen and free carbon dioxide in the culture medium were determined at various densities of population.

The pH, as determined with a Hellige Comparator, never left the range 6.9–7.1, and even within these narrow limits it was not correlated with the population density nor with the length of time that animals had been living in the water. It can be said with a fair degree of certainty that the hydrogen ion concentration never exerted an important influence upon population growth.

The concentration of free carbon dioxide was determined by a titration method reported in a publication of the American Public Health Association (1936). Thus determined, the amount in unused pond water at 18° or at 25° was imperceptible. At the end of a two-day period, crowded populations at 18° had raised the concentration to an average of 4.97 p.p.m.; at 25°, to 5.66 p.p.m.

Winkler Method determinations of the dissolved oxygen concentrations of fresh pond water and water conditioned by large populations for two days yielded the following results: 1. At 18°, unused water 8.38 p.p.m.; after two days' conditioning 6.59 p.p.m. and 2. at 25°, unused water 7.57 p.p.m.; after two days conditioning 4.81 p.p.m. Each of these figures is the average of ten determinations.

There is no evidence available at the present time as to whether or not these slight changes in carbon dioxide and oxygen are sufficient to account for the limitation in population growth. It is also possible that the limiting factor be some metabolite such as that postulated by Brown and Banta (1932) for male production.

COURSE AND ANALYSIS OF POPULATION DEVELOPMENT

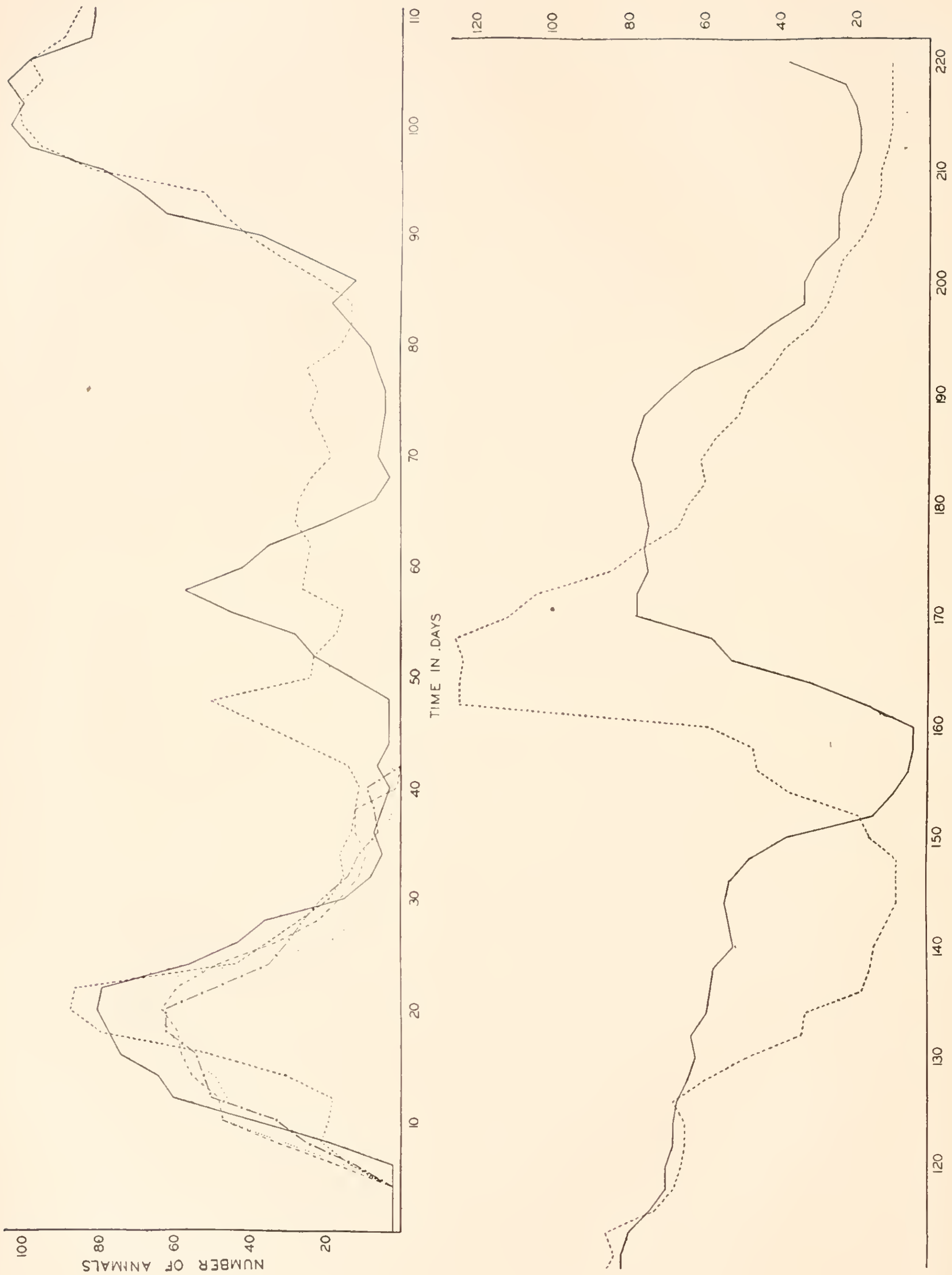
The problem of determining the influence of temperature upon population development resolves itself into two phases: 1. a descriptive study of the observable effects of temperature upon the form and dimensions of the population curve, and 2. an analysis of the processes through which the difference in temperature brings about the observed results. The present section is confined to the presentation of the factual data on the history of populations at 18° and 25° and the discussion of these growth curves. The analysis of the influence of temperature will be dealt with in later sections.

A. *Observations at 25° and at 18°.*

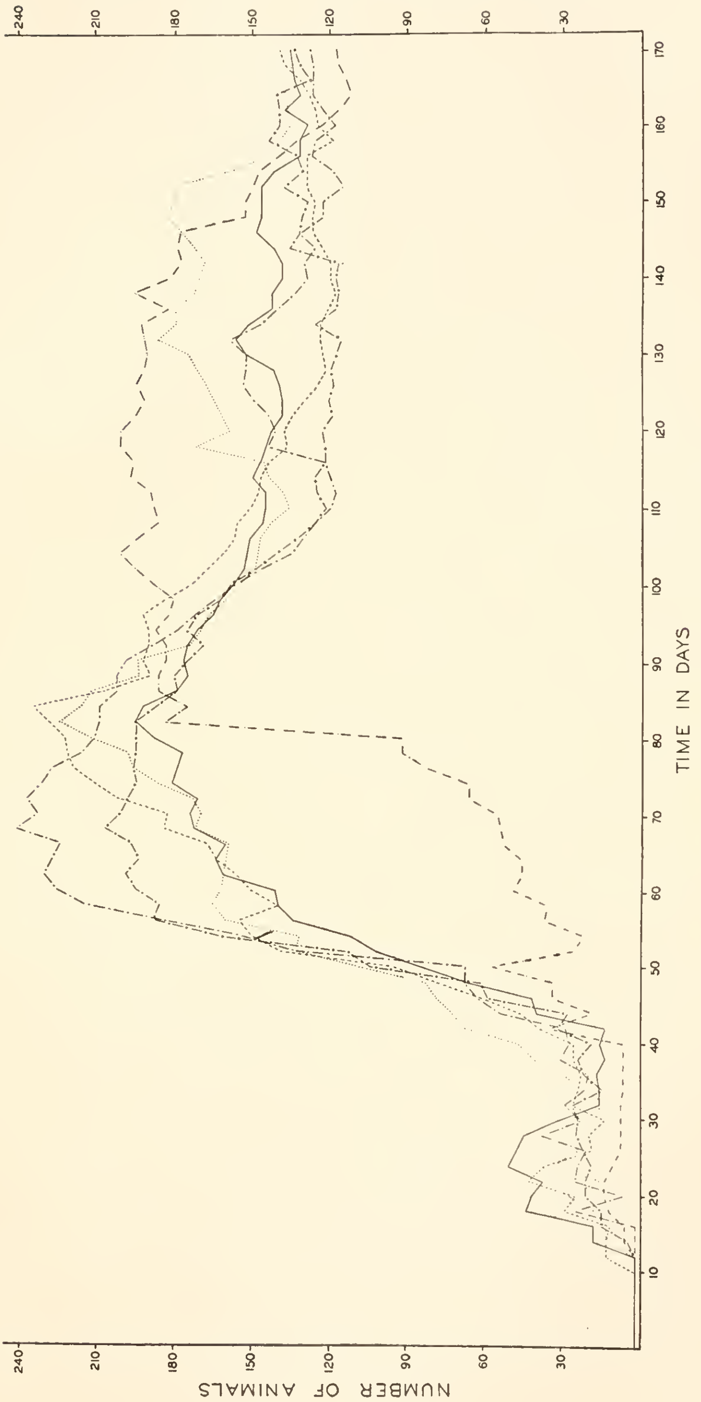
At 25°, four series of populations were started on different dates in January and February 1942. The histories of these 21 populations were recorded either until their natural extinction or until September 13, 1942, when all remaining populations were discontinued. Graph I presents the observations on a typical series, and reveals that the 25° population curve is characterized by violent and fairly regular oscillation. Instead of terminating in an upper asymptote, the first period of increase results in a pronounced peak, after which the curve drops almost to the baseline, then repeats the cycle. Typically there is no asymptote.

The majority of the populations became extinct before the experiment was terminated. Those that survived until the 234th day, when observations ceased, described, commonly, four major oscillations in numbers. The maximal size attained was a population of 126 animals.

At 18°, three series of populations, started on different dates in late March 1942, were followed until September 13, 1942, when the experiment was terminated. None of these 16 populations became extinct in the 174 days of observation. Graph II, presenting the histories of a typical series, shows that each curve described a prominent peak, followed by a gradual decrease and virtual stabiliza-



GRAPH I. Population development at 25° C. (Series C.)



Graph II. Population development at 18° C. (Series C.)

tion or continued oscillations of relatively minor amplitude. The greatest maximum achieved was 241 animals in the 50 cc. environment, and after the major increase, a density of more than 100 animals was permanently maintained in all the populations. While the course of development at 18° is oscillatory, it differs from that at 25° in various points that will be examined later.

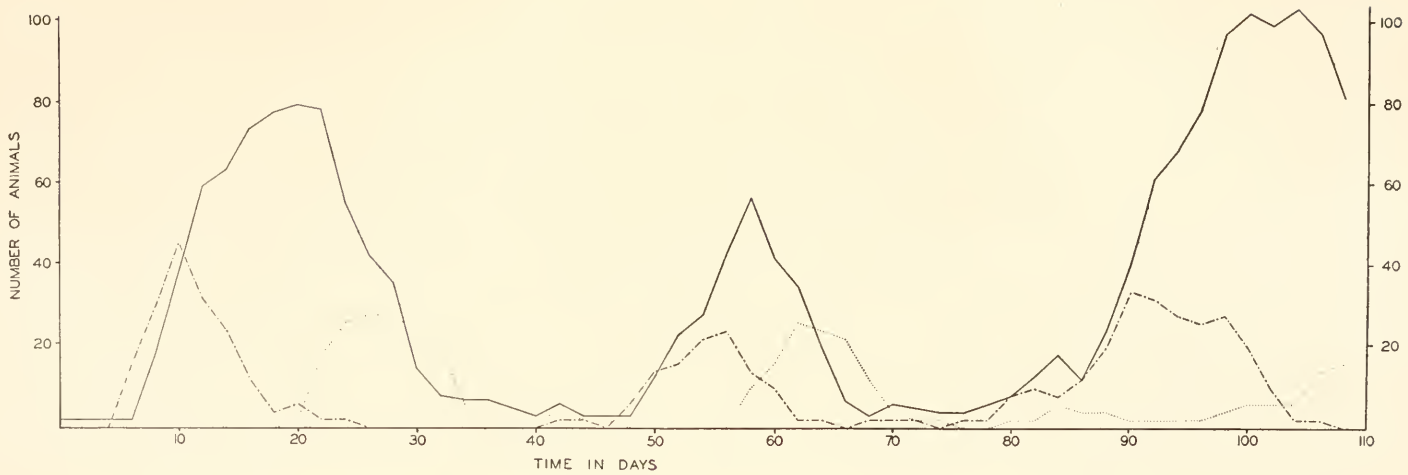
Preliminary experiments in which the volume of the medium used had been 100 cc. rather than 50 cc. yielded similar results with regard to the relative shapes of the curves, and population maxima of 381 individuals at 18° C. and 296 at 23° to 24° C.

B. Analysis of oscillation.

Since oscillation is especially pronounced at 25°, the discussion of this phenomenon will be illustrated with the data from that temperature. The analysis of fluctuations in the size of a population is essentially the study of changes in the ratio of births to deaths. Whereas the fundamental feature of an asymptotic population is that at some point birth rate and death rate become equal and thereafter remain constant, in an oscillatory population the curves describing birth rate and death rate repeatedly cross each other and never remain equal. The present fluctuations might be due to oscillation of the birth rate about a constant death rate, or to the converse, or to differential changes in both rates. In order to establish the cause of the fluctuations, it is therefore necessary to ascertain by which of these three methods the ratio of births to deaths varies.

Daily tabulation of births and deaths revealed that the oscillations observed in the 25° populations resulted from changes in both the number of births and the number of deaths. Periods of increase in population size were marked by a combination of high reproductive activity and low mortality; decreases were caused by increased mortality coupled with negligible reproduction. This mechanism of oscillation, in terms of the changing births/deaths ratio, is illustrated in Graph III which depicts part of the history of a representative 25° population (No. 2 in Graph I), with curves showing the numbers of births and deaths for each day of population census.

The history of one complete cycle will illustrate the reasons for these changes in the ratio of births to deaths. At the outset of an upward swing, the population consists in a few adults. Having lived the greater part, if not the whole, of their lives under favorable environmental conditions as regards crowding, these individuals exhibit a high reproductive rate. The growing population is composed of a few (two to 10 or 15) rapidly reproducing adults and their much smaller offspring. Graph III shows that the increasing population density begins to exert its harmful effects upon the reproductive rate before it affects the death rate, as it does in growing populations of Drosophila (Pearl, 1927). Thus the reproductive activity of the few adults in the population gradually dwindles, and the population reaches the maximum. The crucial and distinctive crossing of the birth and death curves at about this point is ascribed to two factors: 1. the extent of biological conditioning that occurs in the 48 hours between changes of the medium is presumably greater at this density than ever before, and 2. the cumulative adverse effects of crowding upon animals that have lived the greater part of their lives at high population densities begin to manifest themselves. The effect of these factors is sudden and severe: the death rate soars and reproduction is greatly



Graph III. Mechanism of oscillation at 25° C.

Legend: population size ————————
deaths * . . . .
births * —·—·—·—·—·—

* If the actual number of deaths and births occurring on each day is plotted, the resulting curves are too irregular and too low to read with ease. Accordingly, each number was doubled, and the curves smoothed by plotting the points as 3-point moving averages.

reduced. The rapidly growing population has "overshot" the density which it might theoretically be just capable of sustaining.

That the population overshoots the density of potential equilibrium does not explain why it drops dangerously close to extinction before recovering itself. At a point, for example, halfway in its descent it might be expected to rally its forces and start up again, since this same intermediate density on the upswing had been correlated with a high reproductive rate and low mortality.

In seeking the reason for the difference in performance of the two populations of equal density, it should be borne in mind that the composition of the waning population at any given point differs significantly from that of the waxing population of the same numerical strength. For example, the average size of the individuals, and hence the total biomass, of the waning population is much the greater. This fact suggests the possibility that the total metabolism of the declining population is higher than that of the growing population. If this is true, the conditioning of the medium by the accumulation of metabolic wastes or depletion of the dissolved oxygen would proceed at a greater rate in the water of the old population than in that of the younger one, and the given numerical density would exert a more severe effect upon the former than upon the latter. This might account for the difference in the subsequent histories of the two populations.

The question of comparative total metabolism was tested by determining the relative rates of depletion of dissolved oxygen in the medium. Fifty adult Daphnia chosen at random from a large culture were placed in each of six bottles with 50 cc. of pond water containing Chlorella. A similar series was made up using smaller animals from the same culture, 50 to the bottle. At the end of two days the dissolved oxygen content of the water was determined by the Winkler method, three bottles being required for each determination. The animals were then transferred to fresh pond water, 50 cc. to the bottle, for a second two-day period, after which the oxygen concentrations were again determined. The average depletion of dissolved oxygen per two-day period of conditioning was 1.19 p.p.m. in the water occupied by the 50 adults, 2.16 p.p.m. in the medium of the 50 young. In so far as the rate of oxygen depletion is a measure of metabolism, the difference between these two figures indicates that the waning population of larger biomass has a lesser, rather than a greater, total metabolism than the waxing population of equal numerical size. Thus the continued decrease of the old population cannot be assigned to a higher rate of conditioning of the medium.

There are two explanations for the continued decrease, the first of which is to be found in changes in the age structure of the population. Since the individuals of the declining population are of a greater average age than those of the increasing population, their life expectancy is of course less. Thus the difference in constitution of the waning population provides a reason for the higher daily number of deaths in this phase of the cycle.

The second reason for the continued decrease in size of the population at densities that formerly permitted increase is disclosed in the study of its previous history as compared with that of the waxing population. The components of the young, growing population had lived all of their lives, up to any density selected for comparison, at population pressures lower than the given density. They had never suffered severe crowding. But the individuals in the waning population of identical numerical strength have lived perhaps their entire lives at densities greater than the present density.

A special experiment demonstrated that the life-long crowding experienced by the latter individuals exerts a permanent adverse effect upon their reproductive capacities. Animals were raised in a crowded condition until their first clutches were laid in the brood chamber. They were then segregated, one animal to a bottle, and their subsequent reproductive rates were compared with those of animals reared in isolation. Unfortunately this early experiment was conducted under conditions slightly different from those obtaining in the present population studies: the volume of water used was 100 cc. rather than 50; and the temperature, not controlled, varied between 22° and 27° C. The "crowded" state was a population density of 25 animals/100 cc. Animals that lived under these conditions as young (i.e. until the sixth day) and then were segregated, each into 100 cc., exhibited a reproductive rate only 62.7 per cent of that of animals that had never suffered crowding.

It should be noted that this drastic effect was brought about by crowding of only a very moderate intensity, as compared with that experienced by the declining populations under discussion. Individuals whose previous history of crowding has included population pressures ranging from 60 to 120 animals/50 cc. no doubt suffer a far more severe inhibition of reproduction. Furthermore, it is believed that subjection to high densities during early life has a lasting deleterious effect upon survival as well as reproduction, which would help to explain the persistence of a high death rate as well as a low birth rate in the shrinking population.

The effects of previous crowding may be sufficiently severe and persistent to inhibit reproduction in the waning population completely and permanently. In this event the population becomes extinct. As a rule, however, a few young are produced toward the close of the cycle, pass their juvenile stages at minimal population pressures, and attain maturity with their reproductive capacity unimpaired by crowding. From these animals stems the next growth cycle of the population.

Oscillation consists essentially in the successive "overshooting" and "undershooting" of a theoretical equilibrium density. These phenomena appear to be due to a delay, rather than a prolongation, in the manifestation of density effects. The growing population withstands a high degree of crowding with a negligible mortality. There is a lag before the effect of these population pressures is fully felt upon the death rate, which, once raised, remains high for several days while the decreasing population passes through formerly favorable densities. Likewise the reproductive rate of the increasing population is at first unaffected by high densities, but when finally checked, does not recover from the effects of crowding until long after that state of crowding has ceased to exist. Thus overshooting is occasioned by a delay in the expression of the adverse effects of high densities upon reproduction and mortality, and undershooting results from a similar lag in the manifestation of the beneficial effects of favorable densities.

C. Comparison of Oscillation at 25° and 18°.

The principal point of contrast in form of population curve at the two temperatures is the continued oscillation at 25° as compared with the tendency of the 18° curves to approach an equilibrium value.

Table I presents the duration, size-range, and mean size of the equilibria established at 18° and at 25°, and the days of the respective population histories that bounded these equilibria. After a single peak ranging from 184 to 241, oscillation at 18° was greatly reduced in all cases, six of the populations achieving nearly constant values (averaging 135.4) that they maintained until observations ceased, whereas at the higher temperature equilibria were established on only three occasions, and the general course of development was marked by a steady increase, rather than a diminution, in both the amplitude and the period of oscillation.

This progressive increase in the magnitude of oscillation at 25° is correlated with a noteworthy decrease in the rate of population extinction. Of the 21 original populations, only seven survived until the third oscillation, but of these, six were still flourishing when the experiment was discontinued. The reason for the improved adjustment or heightened resistance to the environment apparent in the latter half to two-thirds of population history at 25° is not clear.

TABLE I

Population equilibria at 18° and at 25° C.

Temperature	Population	Graph	Days bounding equilibrium	Duration	Size range	Mean size
25°	Series A, No. 3	*	30–102	72 days	8– 30	21.9
	Series A, No. 3	*	132–234	102 days	30– 54	43.4
	Series C, No. 5	I	50– 80	30 days	15– 28	22.5
18°	Series A, No. 1	*	110–174	64 days	124–145	133.6
	Series A, No. 2	*	96–174	78 days	121–157	140.7
	Series B, No. 5	*	112–172	60 days	127–156	140.4
	Series C, No. 1	II	104–170	66 days	116–144	127.6
	Series C, No. 2	II	100–170	70 days	129–157	143.3
	Series C, No. 5	II	122–170	48 days	118–140	127.0

average of mean values for equilibria at 25° C. = 29.3
average of mean values for equilibria at 18° C. = 135.4

* Graph not presented in this paper.

Barring mutations, one cannot postulate genetic improvement through natural selection, for all the animals were genotypically identical. Since the controlled ecological conditions did not vary throughout the course of the experiment, one is led to suspect some environmental factor that was not controlled. Of these, there is only one which could conceivably have evoked the observed effect. As previously stated, no attempt was made to control conditions of light. The daylight, received from a north window, varied from season to season. It is not improbable that the amount of dissolved oxygen in the medium was an important factor in population growth. The period in which the populations appear to have been better adjusted or more resistant to their environments, beginning at the end of April, coincided with the season in which a longer daily duration of effective light enabled the food-alga Chlorella to produce a greater amount of oxygen. This added daily increment of oxygen may have been sufficient to account for the greater success of the 25° populations in the late spring and summer months. These experiments are to be repeated, at least in part, under more rigidly controlled light conditions.

If this is the correct interpretation of the increase in amplitude and period of oscillation at 25°, evidently the 18° populations were started too late in the season to experience any such improvement in environmental conditions.

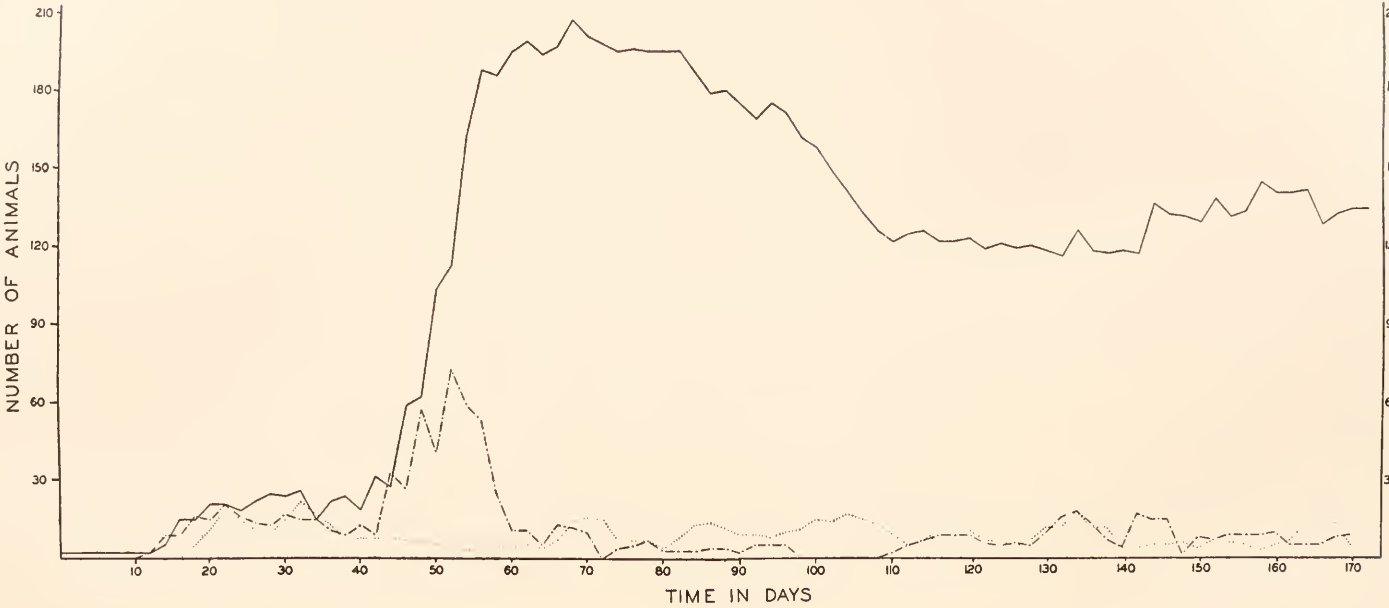
A second point of comparison is found in the nearly complete and simultaneous population "overturns" at 25°, and the more continuous overlapping of generations at 18°. At the higher temperature the first peak in numbers was due entirely to the reproduction of the seed animals, which produced several broods. Typically the first generation animals died during the first population decrease, before the appearance of the third generation. The latter individuals were not produced in numbers sufficient to prevent further decrease. None of the first or second generation animals remained at the inception of the second major increase, which was brought about by the production of the fourth generation. Thus there was a minimum of overlapping of generations. Similar population overturns, more or less complete depending upon the depth and duration of the depressions, occurred between all the subsequent peaks.

In contrast, the course of development at 18° after the major upswing was not thus punctuated by the simultaneous mass replacement of one generation by the succeeding generation. A significant feature of population history at the lower temperature was the accumulation of successive generations. The simultaneous presence of animals of all ages insured a steady replacement of adults and resulted in a sustained continuity in growth and maintenance never observed at 25°.

The lack of a sufficient number of steadily reproducing adults in an 18° population occasions the spasmodic type of population growth witnessed in the first 30 or 40 days of development. This was the period during which the second generation was being produced. The relatively infrequent production of young by the two seed animals and a comparatively high infant mortality result in a highly irregular curve. The attaining of maturity by animals of the second generation caused the tremendous increase in population size which began on about the 40th day. From this point on, the overlapping of successive generations and the constant replacement of producers gives the curve its characteristic unbroken continuity.

It should be recalled that oscillation at 25° results from an alternation of fluctuations, approximately equal in amplitude, in the number of births and the number of deaths per day. The mechanism of oscillation at 18°, in terms of the births/deaths ratio, can be analyzed in Graph IV, which is similar in purpose and in method of construction to Graph III. The data are those of population No. 1 in Graph II.

Examination of these curves reveals that there was far greater variation in the number of births per day than in the number of deaths. Moreover, the two major changes in the size of the population, viz. the tenfold increase between the 40th and 56th days, and the later more gradual decrease, were correlated, respectively, with the periods of maximal and minimal numbers of daily births. While it must be conceded that the number of deaths per day was slightly greater while the population decreased than during the period of increase, both of these levels on the deaths curve are equalled in other parts of that curve, and the difference be-



Graph IV. Mechanism of oscillation at 18° C.

Legend: population size ————
deaths * . . . .
births * —·—·—·—·—

* Plotted as in Graph III.

tween them is immaterial when compared with the variations observed in the births curve. Thus the principal changes in the size of the population are attributable to fluctuations in the number of births, while the number of deaths per day remains approximately constant. This mechanism of oscillation should be contrasted with the alternating fluctuations, approximately equal in amplitude, in the numbers of daily births and deaths that constitute the mechanism of oscillation at 25°.

The lowering of the temperature to 18° exerts a damping effect upon the magnitude and persistence of oscillation that characterizes population development at 25°. At either temperature the waxing population reaches a size that it is incapable of sustaining indefinitely. Whereas at 25° this maximum was generally less than 100 and never exceeded 126, all the 18° populations attained peaks approximately twice as high, covering the range 184–241. At 25° the effects of previous crowding upon both reproduction and longevity manifest themselves quite suddenly, and a sharp peak is described. At the lower temperature crowding acts upon reproduction alone, and its full expression is delayed longer than at 25°, with the result that the population maintains its maximum long enough to describe a short "plateau." Furthermore, whereas the 25° curve sinks almost to the baseline before increase is again possible, the waning 18° population succeeds in halting its decrease at a density which it can maintain, with minor oscillations, for at least 78 days. It should be noted that this density is greater than that achieved in any of the 25° maxima. Thus regardless of temperature, the waxing population overshoots the density of potential stabilization, but the waning population at 18° does not undershoot it as the 25° population does. This virtually terminates oscillation at the lower temperature after the first peak, in sharp contrast to the continued and progressively increased oscillation at 25°.

Influence of Temperature upon Longevity and Reproduction

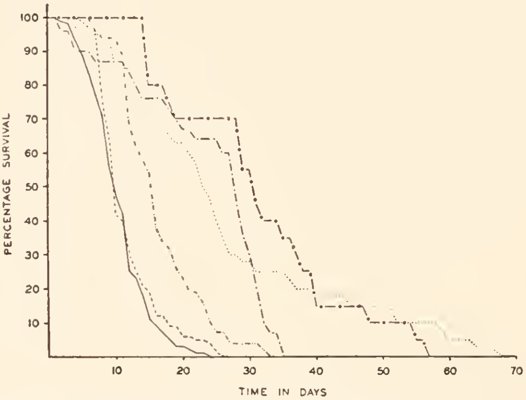
To account for the observed differences in the histories of populations at 18° and 25°, a series of experiments was undertaken to determine the effect of these temperatures upon the two primary variables in population growth, namely duration of life and the reproductive rate. Since such an investigation must take into consideration the influence of population density if it is to accomplish its ultimate purpose, the experiments were so designed as to measure, at each of the temperatures tested, the mean longevity and reproductive rate of animals living at different constant densities.

The desired number of parthenogenetically produced female Daphnia were placed in 50 cc. of fresh pond water with Chlorella added, not more than 12 hours after their release from the maternal brood pouch. The medium was renewed at two-day intervals, and the dead animals and young, when they appeared, were removed and counted daily. Population pressures of more than one animal per bottle were maintained constant by the introduction of substitute animals to take the place of those that had died. The problem of distinguishing these "substitutes" from the extant original members of the controlled population was surmounted by staining them with Neutral Red, a vital stain which in concentrations sufficient to dye the animals apparently did not injure them. (When fed only Chlorella, *Daphnia magna* does not develop the rich red color generally characteristic of the species.) A staining period of 12 to 24 hours in pond water tinged with a few drops of a concentrated Neutral Red solution rendered the animals distinguishable from untreated individuals for several days. Two precautions were exercised in the selection of substitutes: 1. they were matched for size with the original members still living in the population, and 2. only individuals without eggs were chosen for this purpose. Thus, in so far as it was possible to estimate it, the substitutes' contribution to the total density effect was proportional to their number, and all of the young produced in the population were born of charter members.

A. Experiments at 25°.

Thirty tests were made at a density of one animal per 50 cc., four at densities 5 and 10, and two at densities 25, 50 and 75.

The survival curve of the 30 single animals and the average survival curves for the five higher densities are plotted on Graph V. To facilitate a quantitative comparison of survival at the six densities tested, the total number of animal days lived by each population was divided by the number of original members to give the mean duration of life at each population pressure. These data are plotted on Graph VI, which discloses the noteworthy fact that the greatest mean longevity occurs in populations of five, rather than at the minimal density, and that animals even at a density of ten per bottle lived longer, on the average, than did those in isolation.

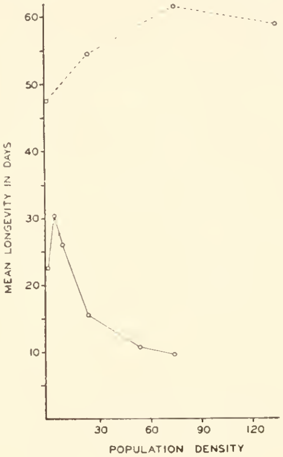


Graph V. Survival at different constant population densities, 25° C.

Legend: population density 1 —·—·—·—·
population density 5 -o-o-o-o-o
population density 10 ··········
population density 25 —··—··—··—
population density 50 ———————
population density 75 ————————

Two interpretations of this phenomenon suggest themselves. MacArthur and Baillie (1929) have developed the thesis that the mean longevity of *Daphnia magna* is an inverse function of the metabolic rate and have reported (1929a) that metabolic rate as indicated by the rate of heart beat is inversely proportional to population density in the range 1 to 25 animals per 100 cc. These authors did not determine the influence of population density upon longevity in Daphnia, but they implied that the reduced metabolic rate evinced by crowding might exert the same effect upon longevity as a metabolism lowered by some other process, such as decreasing the temperature. According to this theory, then, increased population pressure, up to the point of actual injury, might be expected to prolong life. MacArthur and Baillie's hypothesis may give the correct interpretation of the occurrence, observed in the present experiments, of the maximal longevity at a supraminimal density.

There is, however, a second possible explanation for this phenomenon. It was noted that the water in bottles containing only one animal was usually slightly clouded with bacteria, whereas the medium of larger populations was always kept clear by the feeding animals. The bodies of the isolated individuals, when found dead, were frequently covered with a bacterial slime, which was occasionally observed even before death, in severe cases greatly hindering the animal's movements or even imprisoning it completely. The slime was composed of motile rods and spirilla—common fresh water saprophytes—and their gelatinous secretion. Since its appearance upon a live animal in macroscopic proportions almost invariably signalled the death of the animal within a day or two, it is believed to have contributed to the relatively high death rate at the minimal density. More crowded populations apparently never suffered from this effect; their greater numbers enabled them to maintain control of the bacterial flora.



Graph VI. Population density and mean longevity.

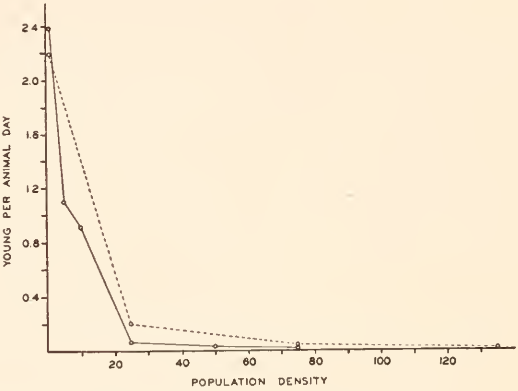
Legend: 18° C. - - - - - - - -
25° C. ————

This explanation is reminiscent of one proposed to account for a similar relation between density and mean longevity observed in populations of a different animal. Allee (1931) has suggested that the positive correlation of mean longevity with population pressure in Drosophila in the density range of 1 to 35 or 55 flies per one ounce bottle, reported by Pearl, Miner and Parker (1927) may be due to the inability of the smaller populations to keep in check the "wild" yeasts contaminating the cultures.

The available evidence does not warrant a decision between the two interpretations, metabolic and bacterial, of the results recorded here. It is not improbable that they are both operative in the present case.

The reproductive rate of each population was calculated by dividing the total number of young produced by the number of animal days lived. Graph VII, in which the results are presented, shows that reproductive rate is an inverse function of density throughout the range in which it was determined, and it drops most rapidly as the density is increased to 25 animals per bottle.

These experiments, yielding quantitative measurements of the effects of various constant densities upon longevity and reproductive rate, offered an opportunity for studying the nature of the density effect as the limiting factor in population growth. In population studies with different animals a variety of density effects have been described, but almost certainly the only influence crowding can exert upon either the birth rate or the death rate of a parthenogenetic form such as Daphnia is through the conditioning of the medium. This might lead one to expect that medium which had been heavily conditioned would exert the same adverse effects upon reproduction and longevity as those brought about by actual crowding. To test this, individual Daphnia were reared in isolation in the water conditioned by the populations of different constant densities. Every other day their medium was renewed with that which one of the larger populations had been conditioning for the past two days. Contrary to expectation the isolated animals suffered thereby no impairment of reproductive capacity. Thus the conditioning of the medium is only temporary, and probably consists in the accumulation of some volatile inhibitory substance, such as carbon dioxide, or the depletion of the dissolved oxygen supply.



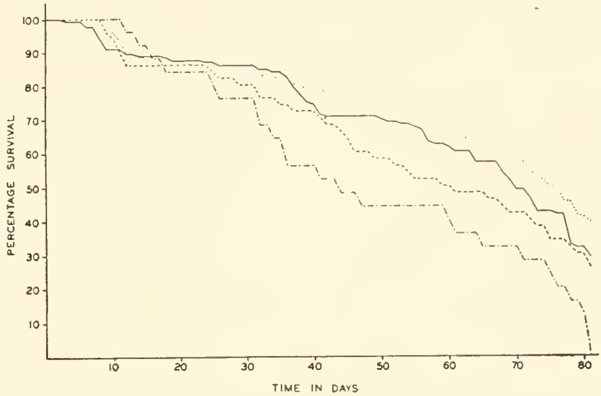
Graph VII. Population density and reproductive rate.

Legend: 18° C. -------
25° C. ———

Further experiments have been planned to ascertain more exactly the nature of this limiting factor. Moreover, individual Daphnia are to be raised in medium effectively connected with that of animals living under crowded conditions, so as to test the possibility of a density effect induced directly by crowding *per se*, rather than indirectly through the conditioning of the medium.

B. Experiments at 18°.

Twenty-five tests were run at a density of one animal per 50 cc., two at densities 5 and 10, and one at density 135. On the 81st day of observation, when these experiments had to be discontinued for lack of time, all the animals at the minimal density were dead, but some individuals were still living at each of the three higher densities. The data are therefore complete for density 1 but must be regarded as partial only for densities 25, 75, and 135. Had the experiments gone to completion, the mean longevities for the latter three densities obviously would have been higher than those obtained, and it is impossible to say whether the average reproductive rates would have differed appreciably from those observed, and in which direction. Of the two sets of data, those concerning reproduction may perhaps be considered the more accurate.



GRAPH VIII. Survival at different constant population densities, 18° C.

Legend: population density 1 —·—·—·—·—
population density 25 – – – – – – –
population density 75 · · · · · · · · · ·
population density 135 ————————

The range of densities tested was extended to include the mean equilibrium value, 135 (see Table I, p. 126), in the 18° populations. The survival curve of the 25 single animals and the average survival curves for the other densities are plotted on Graph VIII.

Calculation of the mean longevity by dividing the total number of animal days by the density of the population yields results which, for densities 25, 75 and 135 are obviously only minimal, since some animals were still living at those densities when observations ceased. The data are shown in Graph VI.

The mean reproductive rates for the 81 days of observation are presented in Graph VII.

C. Comparison of results, 25° and 18°.

Throughout the range of population densities tested, duration of life is greater at 18° than at 25° (Graph VI). This results from the positive correlation of metabolic rate with temperature and the negative correlation of longevity with metabolic rate.

The action of population pressure is quite different at the two temperatures. Whereas a density of 5 was found to be the optimum for duration of life at 25°, throughout the density range 1 to 75 longevity at 18° is a direct function of population pressure, and animals living at density 135 lived, on the average, longer than did those in isolation. The bacterial contamination of the medium which is believed to have contributed to the death rate of single individuals at 25° was never observed in the 18° bottles. This is, then, an indirect effect of temperature upon duration of life: even at the minimal density the colder water did not support a bacterial flora sufficiently concentrated to injure the animals. The absence of a bacterial effect at 18° makes it seem likely that the positive correlation of density and longevity in this case is incidental to a lowered metabolic rate. At 18° the population pressure at which metabolism is depressed to the point of positive injury lies somewhere in the density range 25 to 135, perhaps at about 75 animals per bottle.

Whereas a decrease in temperature of 7° raised the mean longevity, presumably through depression of the metabolic rate, it did not thereby bring about an equivalent reduction in reproductive rate. Apparently the rates of reproduction and mortality are not dependent upon exactly the same physiological processes. If they were, a given increase in longevity with a reduction in temperature would be correlated with a decrease in birth rate of the same magnitude.

The action of increasing density upon reproductive rate is very similar at the two temperatures (Graph VII), although it is slightly more severe at 25°: at the minimal density birth rate at 25° is higher than at 18°, but drops faster with increased crowding and at density 25 is slightly lower than the corresponding 18° rate.

The relative potential rates of population increase (i.e. the rates that would obtain if there were no density effects) can be calculated by comparing the data for the minimal density at the two temperatures. The birth rate at 18° is 2.19 young per animal day, or a gross factor of daily increase of 2.19X. The death rate (which is the reciprocal of the mean longevity, or 1/47.6) is .021X per day. Thus the net rate of potential daily increase (2.19X–.021X) is 2.17X. At 25°, gross increase (2.38X) minus death rate (.044X) yields a net rate of potential daily increase of 2.34X. When one considers that birth rate at 18° is only slightly less than at 25° and that longevity at 18° is more than twice as great as at 25°, this result is perhaps astonishing, but it illustrates the fact that birth rate is so much greater than death rate as to be the only effective factor in the net rate of increase. The reproductive rate taken alone gives the 25° population an initial advantage of .19X (2.38X–2.19X) over the 18° population. Granting the observed 25° death rate of .044X, the net rate of potential increase at 18° could not equal that at 25° even if death rate at the lower temperature were reduced to zero. But, as we have seen, the differential action of population density is such that ultimately a population attains a greater size at 18° than at 25°.

It will be recalled that oscillation at 25° was brought about by an alternation of approximately equivalent effects of population density upon the number of births and the number of deaths. The results of the reproduction and longevity experiments at 25° bear out the contention that population density at that temperature affects both of the primary variables in population growth, in opposite directions and to approximately the same extent. On the other hand, the reproduction and longevity experiments at 18° give results consistent with the observation that the mechanism of oscillation at that temperature was the fluctuation in the number of births about a nearly constant daily number of deaths. Population pressure has a relatively insignificant effect upon mortality. Moreover, if the number of deaths per day in an increasing population remains constant, the death *rate* must be an inverse function of population density. This deduction is supported by the fact that mortality in the reproduction and longevity experiments was lower at density 135 than at the minimal population pressure. This action of density upon death rate, operating in generally the same direction as the effect of density upon birth rate, tends to moderate, rather than intensify, the severity of oscillation. Therefore oscillation at 18° must be attributed wholly to changes in birth rate.

From the reproduction-longevity data one can calculate theoretical asymptotes for populations at 18° and at 25°. The number of young produced by an individual of mean longevity and reproductive rate at a given density can be determined by dividing the total number of young born at that density by the size of the population. These figures for the four population pressures tested at 18° C. are as follows:

density	1	25	75	135
average number of young individual	104.5	10.7	0.73	0.19

Obviously a population of such density that each member could just replace itself before dying should be capable of maintaining a constant size. It is found by interpolation that the density at which the average animal produces one young in the course of its life is 73.6. It should be noted, however, that this theoretical asymptotic value is considerably lower than the mean of equilibrium values (135.4) actually established in the 18° populations. The discrepancy is serious, and perhaps cannot be entirely explained by the fact that the reproduction-longevity experiments did not go to completion.

At the higher temperature the agreement between observed equilibrium values and the theoretical asymptote is much closer. The number of young produced per individual in the 25° reproduction-longevity experiments is as follows:

density	1	5	10	25	50	75
average number of young individual	53.9	26.7	23.1	0.82	0.26	0.08

The calculated asymptote is 24.8 animals per bottle, while the mean of equilibrium values actually observed in the 25° populations is 29.3.

The explanation for the discrepancies between observed and calculated equilibrium values is not clear. It is suggested that the age-structure of the population is a significant factor. Apparently the conditions implied by a density of a given number of animals of the same age are different from those implied by a density of the same number of animals of different ages. Although the reproduction-longevity experiments at both temperatures yield theoretical equilibrium values that are probably lower than the actual levels of stabilization, these experiments undoubtedly give a faithful picture of the relative effects of different densities upon the reproductive rate and upon longevity, and the data for the minimal densities can be regarded as absolute, under the given conditions.

Discussion

A. Oscillation.

Fluctuations in the density of populations in nature can usually be assigned to changes in environmental forces, which may be physico-chemical or biotic. The environmental disturbance may evoke an immediate response in the numbers of the species under consideration (as in the case of epidemics, sudden changes in meteorological conditions, etc.), or its action may be delayed for a longer interval. An example of this second category is the determination of the future size of adult populations of marine fishes by the effects of various environmental agencies upon the early developmental stages (Hjort, 1914; Johnstone, 1928). Presumably the periodic oscillations in the numbers of fur-bearing mammals and game birds which have been synchronized with sunspot cycles (Elton, 1924; Gross, 1931; Naumov, 1939; Braestrup, 1940; Green and Evans, 1940) result from complexes of environmental vectors whose action is more or less delayed.

When one considers the instability of the environment, it is not surprising that natural populations undergo violent fluctuations. However, it has been argued from mathematical grounds that the interaction of two or more animal species, e.g. predator and prey (Volterra, 1926) or parasite and host (Nicholson, 1933) is such as to give rise to rhythmic pulsations in the numbers of the animals, even though the environment is maintained constant in all other respects. In such a situation, oscillation in population density would be attributable to biotic forces exclusively, the physico-chemical factors of the environment being fixed.

Of the cases of fluctuations in numbers whose cause has been ascertained, all that have come to my attention are laid to variations in some external agency. The oscillations in Daphnia populations discussed in this paper are of an essentially different nature. Here the agent of fluctuation is internal and intrinsic. While the environment plays an important role, it is an environment whose critical changes are determined by the activities of the animals themselves. The cause of oscillation is the delay in the action of population density upon mortality and the reproductive rate, rather than a variation in some external environmental agency. It is obvious that fluctuation would not occur if the effects of a given density upon birth and death rates manifested themselves immediately; an increasing population would gradually develop an asymptote instead of "overshooting." Thus the ultimate source of oscillation is a lack of synchronization of a physiological state with the forces that provoke it.

B. Influence of temperature on population size.

It was originally intended to obtain a quantitative expression of the influence of temperature on population size by comparing the asymptotes developed at the different temperatures. Since this is clearly impossible, apparently the most satisfactory comparison would be one involving the mean sizes of the populations.

The average sizes of the 21 populations at 25° covered a range of 18.5 to 43.0, with the average at 32.6. The range of average sizes of the 16 populations at 18° was 104.7 to 126.2, the mean 112.4. Thus the mean of population size at 18° was about two and one half times as great as at 25°.

This result is consistent with the common experience that populations, and particularly those of marine plankton, attain greater densities in cold than in warmer regions (Oltmanns, 1923; Bělehrádek, 1935; Welch, 1935; Russell and Younge, 1936; Hesse, Allee and Schmidt, 1937). It should be borne in mind that this greater abundance in polar waters refers to the size of the equilibrium population, rather than to the productivity in terms of the rate of turnover.

Inasmuch as the cause of this relative abundance is still not certain, and a variety of theories have been developed to account for it, the possibility of applying the results of the present study to the problem should be of considerable interest. At the outset, however, it is apparent that the type of environmental factor preventing unlimited growth in these experimental populations (i.e. a biological conditioning of the medium by the accumulation of metabolites and/or depletion of the oxygen supply) is probably never an effective limiting factor in the open ocean. In recent years, however, it has been contended that the latitudinal variation in plankton abundance so often observed in the sea obtains in fresh waters as well (Welch, 1935), and it is quite possible that the limiting factor in the Daphnia populations is operative in some fresh water situations.

Of the various hypotheses advanced by the oceanographers, there is but one which might be applicable to the present case. This is the theory that attempts to explain the greater asymptotic level of polar planktonic populations by a direct effect of temperature upon the metabolic rate. It is argued that the lower metabolism in cold waters results in a longer duration of life and thus in an accumulation of generations; and further, that this increase in longevity more than offsets the concomitant reduction in reproductive rate. In short, the decrease in temperature exerts a greater effect on duration of life than upon the birth rate. Loeb (1912) supported this argument with the observation that the prolongation of life of sea urchin eggs with a drop in temperature greatly exceeded the retardation of their development. The theory involves only the *direct* effects of temperature upon birth and death rates. It alleges to explain the observed results without reference to any action of population density upon reproduction and duration of life.

A critical examination of the Loebian theory discloses that it really cannot account for differences in asymptotic levels. The disproportionately greater longevity at the lower temperature cannot possibly influence the height of the asymptote, since birth and death rates in a population that has attained an asymptote are equal. The equilibrium level is determined by two factors: the previous rate of increase of the population and the duration of that increase. The birth rate/death rate ratio determines the rate of population increase. It is in this ratio that the disproportionately great longevity at the lower temperature would express itself, yielding a greater net rate of population increase in colder than in warmer waters. But the second factor, namely the duration of population increase, is in no way affected by the birth rate/death rate ratio. It is determined by some limiting factor in population growth other than temperature. This limiting factor may, in turn, be influenced by temperature, but it is essentially a result of population density. Without it, that is with no limit to the duration of increase, the population would continue growing, geometrically and indefinitely. Since Loeb's theory involves only the rate of increase and disregards the factor of duration of increase it makes no provision for any check in population growth. The inescapable conclusion is an everlasting logarithmic increase. From a slightly different point of attack this criticism may be rephrased thus. Since there are two factors involved in the asymptotic level ultimately attained, a population with an infinitesimally low rate of increase may eventually reach a greater asymptote than that developed in shorter time by another population with a much higher rate of increase. As an example, in the present experiments the potential rate of increase at 25° was found to exceed that at 18°, yet populations at the latter temperature attained the greater mean size. It should be pointed out, however, that owing to seasonal phenomena, the time element may be critical in the development of some populations in nature. Because of the brevity of the favorable season, these populations may never reach their potential asymptotes. In this case the rate of increase is the all-important factor in the size of the population at any given moment.

The explanation for the greater mean size of the 18° populations would appear to be a differential action of density at the two temperatures. The reproduction-longevity experiments revealed that increasing population pressure exerts a more severe effect upon the birth and death rates at 25° than at 18°. Possibly this result is related to the difference in solubility of atmospheric oxygen in the medium at different temperatures, but whatever the nature of the conditioning may be, the influence of temperature upon mean population size is indirect. It operates through the differential effects of population pressure. Thus the difference in temperature exerts its observed influence upon the mean of population size only by modifying the action of population density.

SUMMARY

1. The development of populations of *Daphnia magna* was followed at two different constant temperatures. Sixteen populations were maintained at 18° and 21 at 25° C. The 50 cc. of pond water which served as medium were renewed every other day and always contained an excess quantity of the food-alga Chlorella.

2. Population development at 25° proved oscillatory in nature, four peaks occurring in 234 days, with a maximum population size of 126 animals. In the 174 days of observation at 18°, one major peak was observed (maximum 241) followed by a decrease and virtual stabilization at a population density of about 135.

3. Analysis of the oscillation disclosed that it is due to a delay in the expression of the effects of population density upon birth and death rates.

4. The mechanism of oscillation at 25° is an alternation of fluctuations in numbers of births and numbers of deaths. The mechanism at 18° is the fluctuation in the number of births about a nearly constant number of deaths.

5. Experiments with a series of population densities artificially maintained constant showed that birth rate at 25° is an inverse function of population density. At 18° the effect of density is similar but less severe.

6. Under these conditions of constant density, mortality at 25° is in general a function of population density, although the minimal mortality occurs at a

density of 5. At 18° mortality is but little affected by conditions of density, and is apparently least at about 75 animals/50 cc.

7. The mean of population size at 18° was two and one half times as great as that at 25°.

8. This fact is compared to the supposed greater density of planktonic populations in polar than in tropical waters. The results of this study cannot be applied to the problem of marine plankton abundance since the limiting factor in the present case (the conditioning of the medium by the accumulation of metabolites and/or depletion of the dissolved oxygen supply) is presumably never operative in the ocean, although it may be operative in some fresh water situations.

9. The possibility of accounting for the greater mean size of the 18° populations by reference to the direct effect of temperature upon longevity is considered but rejected. A basic fallacy is pointed out in the theory which attempts to explain by such a direct effect of temperature the greater density of asymptotic populations in polar than in tropical regions.

10. It is concluded that the influence of temperature upon mean population size observed in these experiments is indirect: the temperature difference exerts its effect only by modifying the action of population density.

LITERATURE CITED

ALLEE, W. C., 1931. *Animal aggregations.* New York.

American Public Health Association, 1936. Standard Methods for the Examination of Water and Sewage. (8th ed.) New York.

BĚLEHRÁDEK, J., 1935 *Temperature and living matter.* Berlin.

BRAESTRUP, F. W., 1940. The periodic die-off in certain herbivorous mammals and birds. *Science,* **92**: 354–355.

BROWN, L. A., AND A. M. BANTA, 1932. Sex control in Cladocera. VII. *Physiol. Zool.,* **5**: 218–229.

CHAPMAN, R. N., 1928. The quantitative analysis of environmental factors. *Ecology,* **9**: 111–122.

CLARK, N. A., 1922. The rate of formation and the yield of yeast in wort. *Jour. Phys. Chem.,* **26**: 42–60.

ELTON, C., 1924. Periodic fluctuations in the numbers of animals: their causes and effects. *Brit. Jour. Exp. Biol.,* **2**: 119–163.

GAUSE, G. F., 1932. Ecology of populations. *Quart. Rev. Biol.,* **7**: 27–46.

GREEN, R. G., AND C. A. EVANS, 1940. Studies on a population cycle of snow-shoe hares on the Lake Alexander Area. III. *Jour. Wildlife Management,* **4**: 347–358.

GROSS, A. O., 1931. Ruffed grouse and prairie chicken. *T. Amer. Game Conf.,* **18**: 186–196.

HESSE, ALLEE, AND SCHMIDT, 1937. *Ecological animal geography.* New York and London.

HJORT, J., 1914. *Rapp. Proc. verb. Cons. perm. intern. expl. mer.* Vol. XX, Chapter VI. Copenhagen.

HOLDAWAY, F. G., 1932. An experimental study of the growth of populations of the "flour beetle" Tribolium confusum Duval, as affected by atmospheric moisture. *Ecol. Monogr.,* **2**: 261–304.

JOHNSTONE, J., 1928. On periodicities in the abundance of young fishes in the Mersey Estuary region. *Proc. Trans. Liverpool Biol. Soc.,* **42**: 42–68.

KETCHUM, B. H., AND REDFIELD, A. C., 1938. A method for maintaining a continuous supply of marine diatoms by culture. *Biol. Bull.,* **75**: 165–169.

LOEB, J., 1912. *The mechanistic conception of life.* Chicago.

MACARTHUR, J. W., AND W. H. T. BAILLIE, 1929. Metabolic activity and duration of life. I. *Jour. Exp. Zool.,* **53**: 221–242.

MACARTHUR, J. W., AND W. H. T. BAILLIE, 1929a. Metabolic activity and duration of life. II. *Jour. Exp. Zool.,* **53**: 243–268.

NAUMOV, J. P., 1939. Fluctuations of numbers of hares. *Voprosy Ekologii i Biotsenologii* **5/6**: 40–82.

NICHOLSON, A. J., 1933. The balance of animal populations. *Jour. Anim. Ecol.*, **2**: 132–178.

OLTMANNS, F., 1923. Morphologie und Biologie der Algen. Vol. I. Jena.

PEARL, R., 1925. *The biology of population growth.* New York.

PEARL, R., 1927. The growth of populations. *Quart. Rev. Biol.*, **2**: 532–548.

PEARL, R., AND L. J. REED, 1920. On the rate of growth of the population of the United States since 1790 and its mathematical representation. *Proc. Nat. Acad. Sci.*, **6**: 275–288.

PEARL, R., J. MINER, AND S. PARKER, 1927. Experimental studies on the duration of life. *Amer. Nat.*, **61**: 289–318.

ROBERTSON, T. B., 1921. Experimental studies on cellular multiplication. I. *Biochem. Jour.*, **15**: 595–611.

ROBERTSON, T. B., 1923. The chemical basis of growth and senescence. *Monogr. Exp. Biol.*, Philadelphia.

RICHARDS, O. W., 1928. The growth of the yeast Saccharomyces cerviseae. I. *Amer. Bot.*, **42**: 271–283.

RICHARDS, O. W., 1928a. The rate of the multiplication of yeast at different temperatures. *Jour. Phys. Chem.*, **32**: 1865–1871.

RUSSELL, F. W., AND G. M. YONGE, 1936. *The seas.* London and New York.

TERAO, A., AND T. TANAKA, 1928. Population growth of the water-flea, Moina macrocopa Strauss. *Proc. Imp. Acad.* (Tokyo), **4**: 550–552.

TERAO, A., AND T. TANAKA, 1928a. The influence of temperature upon the rate of reproduction in the water-flea, Moina macrocopa Strauss. *Proc. Imp. Acad.* (Tokyo), **4**: 553–555.

TERAO, A., AND T. TANAKA, 1928b. Influence of density of population upon the rate of reproduction in the water-flea, Moina macrocopa Strauss. *Proc. Imp. Acad.* (Tokyo), **4**: 556–558.

TERAO, A., AND T. TANAKA, 1930. Duration of life of the water-flea, Moina macrocopa Strauss, in relation to temperature. *Jour. Imp. Fisheries Inst.* (Tokyo), **25**: 67.

VOLTERRA, V., 1926. Variazioni e fluttuazioni del numero d'individui in specie animali conviventi. *Mem. R. Acca. Naz. dei Lincei*, Series VI, **2**.

WELCH, P. S., 1935. *Limnology.* New York and London.

RATE OF BREAKING AND SIZE OF THE "HALVES" OF THE ARBACIA PUNCTULATA EGG WHEN CENTRIFUGED IN HYPO- AND HYPERTONIC SEA WATER

ETHEL BROWNE HARVEY

(From the Marine Biological Laboratory, Woods Hole, and the Biological Laboratory, Princeton University)

PROBLEM

Arbacia punctulata eggs, when centrifuged in a mixture of sea water and isosmotic cane sugar solution used in the proper proportion to keep the eggs suspended during centrifugation, break quite uniformly into "halves" of definite size (E. N. Harvey, 1931; E. B. Harvey, 1932–1941). The question arises as to whether the eggs break more or less readily in hypotonic solutions than in sea water, and whether the relative size of the two "halves" remains the same, that is, whether the extra water is distributed equally in the two halves. It has been shown by Lucké (1932b, 1940) that when the eggs are broken into halves in sea water first and the halves are then placed in hypotonic sea water, both halves swell but the heavy (red) half swells a little less than the light (white) half owing to the presence of more of the osmotically inactive material (yolk granules) in this (red) half. The present problem is concerned with centrifuging the eggs *after* they have been swollen in hypotonic sea water. It has been shown (E. B. Harvey, 1941) that the rate of breaking and the relative size of the two halves varies with the amount of centrifugal force used. With a force of 10,000 × g, which I have taken as a standard force throughout my experiments, the white (centripetal) half is slightly larger than the red (centrifugal) half. With a greater force, the red half is larger while the white half is correspondingly smaller. With a smaller force, the red half is smaller than with greater forces, and the white half correspondingly larger. In the present experiments, therefore, a uniform standard force was used, 10,000 × g.

The size of the halves obtained by centrifuging the eggs in hypo- and hypertonic sea water and subsequently returning them to normal sea water was also studied, in order to determine how the normal water balance was regained.

METHODS

Before centrifuging, the eggs from one female were kept for a half hour in 60 per cent, 80 per cent, 100 per cent (control) and 125 per cent sea water, a sufficient time for them to attain equilibrium with the medium. Eggs kept for six hours in the solutions showed no appreciable further change in size. The sugar solutions added to the sea water to keep the eggs suspended during centrifugation, were made up of the same tonicity as the hypo- and hypertonic sea water. The four tubes containing 60 per cent, 80 per cent, 100 per cent (control) and 125 per cent sugar-sea water solutions were all centrifuged at the same time in each experiment;

each tube contained one part of the egg suspension to three parts of the corresponding sugar solution, this being the proper proportion to keep the eggs suspended and free to break during centrifugation. The unbroken eggs in all the tubes come to lie at the same level, so that they are all subjected to the same centrifugal force which, of course, varies with the radial distance of the layer of eggs from the axis of the centrifuge, according to the equation $F = .04 \times R$ (= radius in cm.) $\times$ (R.P.S.)^2. It is necessary to use the eggs from only one animal for one experiment since there is considerable variability in size, segregation of granules and ease of breaking in eggs from different females, but those from one female are remarkably constant in this respect. The eggs were centrifuged for three to six minutes at 10,000 × g, according to the ease of breaking of the particular batch of eggs, and were then placed in dishes of sea water of the corresponding tonicity. The measurements were made with an ocular micrometer and checked in several experiments with a filar micrometer; the figures are accurate to about 0.6 μ. The measurements recorded are the average of ten cells, made with an optical equipment giving a magnification of 400 times; the eggs lay free in the media in Syracuse watch glasses.

The experiments were performed many times with the same general results. The data obtained in a typical experiment are given in Table I A, B, C. The same eggs were used throughout the experiment.

RESULTS

Rate of breaking (Table I A)

The rate of breaking into halves may be judged by the number of broken eggs in comparison with the number of whole, unbroken eggs obtained after centrifuging for a definite time with a definite force. When tubes containing suspensions of eggs in a 60 per cent sea water-sugar medium, 80 per cent, 100 per cent (control) and 125 per cent were centrifuged at the same time, usually for four minutes at 10,000 × g, the degree of breaking increased in the order named. In most of the experiments, practically all the eggs were broken in the 125 per cent medium while very few were broken in the 60 per cent medium. An average experiment (Table I A), gave 10 per cent of the eggs broken in the 60 per cent medium, 20 per cent in the 80 per cent medium, 70 per cent in normal sea water and practically all in the 125 per cent medium. In an experiment where only 50 per cent of the eggs were broken in the 125 per cent medium, none were broken in the 60 per cent medium. The eggs break, therefore, less readily in hypotonic sea water, and more readily in hypertonic sea water, than they do in normal sea water.

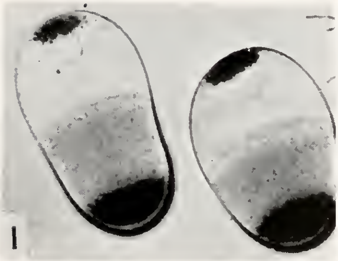
Size of the halves (Table I A; Photographs, Plate I)

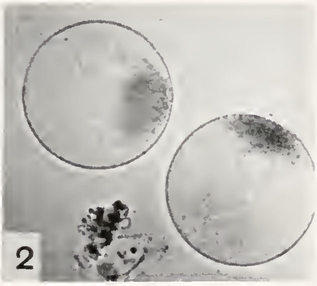
When eggs are swollen in hypotonic sea water or shrunken in hypertonic sea water and then centrifuged, the increase and decrease in size is almost entirely in the white halves, the red halves being nearly the same size as those centrifuged in

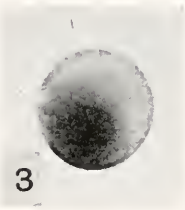
---

PLATE I

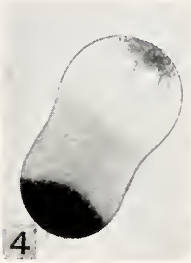
Photographs of living *Arbacia punctulata* eggs centrifuged in hypo- and hypertonic sea water, and the controls in normal sea water, and the halves into which they break with a force of 10,000 × g for four minutes. Magnification approximately 275 ×, all magnified exactly the same.

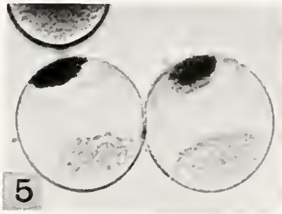

1

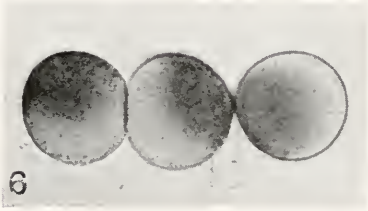

2


3

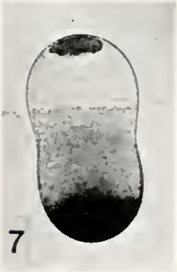
60%

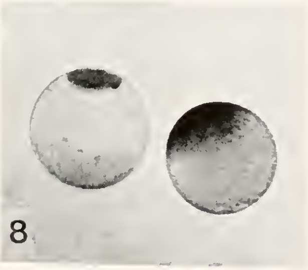

4

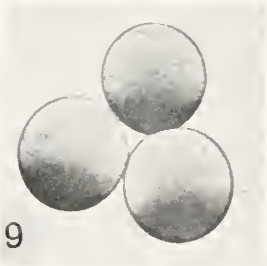

5


6

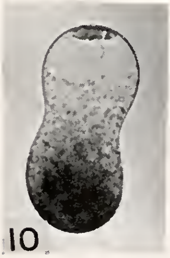
80%

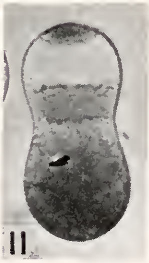

7

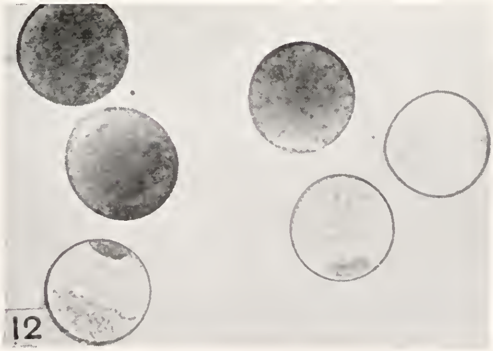

8


9

Control 100%


10


11


12

125%

PLATE I

normal sea water (Table I A). When the eggs are centrifuged in 60 per cent sea water, the white halves are very much larger than those obtained in the control (100 per cent sea water), whereas the red halves are only slightly larger than in the control (Cf. Photographs 2, 3 with 8, 9). When centrifuged in 80 per cent sea water, the white halves are somewhat larger than in the control, the red halves almost the same size (Photographs 5, 6). When centrifuged in hypertonic sea water (125 per cent) the white halves are much smaller than in the control, the red halves about the same size as the controls, sometimes larger, sometimes a little smaller; the white halves are now in most experiments smaller than the red halves (Photograph 12); in a few experiments they were the same size. In the controls, the white halves were always considerably larger than the red halves (Photographs 8, 9).

TABLE I

Sea water	Whole egg		White half		Red half		Nucleus		Per cent broken
	Diam. μ	Vol. μ^3	Diam. μ	Vol. μ^3	Diam. μ	Vol. μ^3	Diam. μ	Vol. μ^3	
A. *Eggs in hypo- and hypertonic sea water, then centrifuged*									
60%	82.4	(292,900)	70.4	(182,700)	58.0	(102,200)	16.0	(2,145)	10%
80%	74.9	(220,000)	62.1	(125,400)	56.3	(93,400)	12.8	(1,098)	20%
100%	72.0	(195,400)	59.0	(107,500)	56.0	(91,950)	11.5	(796)	70%
125%	66.6	(154,700)	51.7	(72,360)	53.8	(81,540)	9.6	(382)	98%
B. *Recovery in 100 per cent sea water*									
60%–100%	72.0[1]	(195,400)	59.2	(108,600)	55.7	(90,480)	11.2	(736)	
80%–100%	72.0[1]	(195,400)	59.2	(108,600)	54.7	(86,170)	11.5	(796)	
100%	72.0	(195,400)	59.0	(107,500)	56.0	(91,950)	11.5	(796)	
125%–100%	72.0[1]	(195,400)	56.3	(93,940)	57.6	(100,060)	11.2	(736)	
C. *Eggs centrifuged in sea water, then placed in hypo-hypertonic sea water*									
100%– 60%	82.4	(292,900)	67.4	(160,300)	63.4	(133,400)			
100%– 80%	74.9	(220,000)	61.8	(123,600)	57.2	(97,990)			
100%	72.0	(195,400)	59.0	(107,500)	56.0	(91,950)			
100%–125%	66.6	(154,700)	54.4	(84,300)	51.2	(70,300)			
D. *Lucké's (1932b)* [2] *mean values for C (above)*									
100%– 60%	(84.6)	317,380	(69.5)	175,560	(63.9)	136,700			
100%– 70%	(80.6)	274,020	(66.3)	152,320	(61.7)	123,060			
100%	(72.2)	197,440	(59.2)	108,600	(55.7)	90,680			

[1] These are not actual measurements because of lack of time to measure these in the same experiment as the rest. The return to normal size is approximately perfect, as determined in other experiments and as found also by Lucké and co-workers, who publish their measurements (1931a, p. 402).

[2] Only the volumes are given by Lucké; the diameters are calculated from the volumes.

Unusual batches of eggs occur occasionally, as noted in previous papers

(1936, 1941), in which, when centrifuged in normal sea water with the standard force, the red half is very small, and the white half correspondingly very large, in the ratio of 8 : 1 by volume. When such batches of eggs are centrifuged in 80 per cent sea water, the halves are of approximately the same relative size as in normal batches, in the ratio of 4 : 3 by volume, as noted previously (1941). When the unusual batches are centrifuged in hypertonic sea water, on the other hand, the relative inequality in the two halves remains; that is, the red halves are very small.

Stratification of whole eggs, and content of halves (Photographs, Plate I)

As would be expected, the granules pack much more when the eggs are centrifuged in hypotonic media than in normal sea water (Photographs 1, 4, 7). The packing of the granules takes place to such an extent in the 60 per cent sea water that the clear layer is very large and usually the white halves (Photograph 2) are almost entirely free of granules, all of them having been thrown down into the red half, although, as stated above, this red half is not much larger than the red half obtained in normal sea water which contains none of the mitochondria and only part of the yolk. This can be beautifully demonstrated in eggs stained with the vital dye, methyl green, which selectively stains the mitochondria. The purple-staining mitochondria are all in the red half. In the whole egg centrifuged in 60 per cent sea water, the mitochondrial layer is very thin, being spread over a greater area. The pigment granules are so well packed in the hypotonic solutions that the line of demarkation between yolk and pigment is very sharp, much more so than in eggs centrifuged in normal sea water. When the eggs are centrifuged in 60 per cent sea water, many of the white halves and also the upper portion of the whole eggs containing the clear layer burst soon after centrifugation; the red halves and red portion of the whole egg remain intact. This bursting is probably due to the thinness of membrane which presumably decreases in thickness as the area it covers increases.

When centrifuged in hypertonic sea water, the clear layer is small, the mitochondrial layer very thick, being spread over a small area, and in many cases is very well marked (Photograph 11). The white half is thus quite granular. The pigment is not well separated from the yolk, there being no clear line of demarkation. It is obvious from Photograph 11, that it is in general not accurate to speak of "well-stratified" eggs, since they may be well-stratified with respect to the mitochondria and poorly stratified with respect to the pigment and yolk. Many batches of eggs occur, in which, when centrifuged in normal sea water, the mitochondrial layer is indistinguishable while the yolk and pigment layers are well formed. In typical batches of eggs, however, the stratification in normal sea water is intermediate between that obtained in hypotonic sea water and in hypertonic sea water (Photograph 7).

Recovery in normal sea water (Table I B; Photographs, Plate II)

When whole normal eggs are swollen in hypotonic sea water or shrunken in hypertonic sea water, and are then returned to normal sea water, they return to normal size, as shown previously (for hypotonic) by Lucké and co-workers (1931a; 1932a). The same holds for centrifuged whole eggs. The two half-eggs obtained by centrifuging an egg swollen in hypotonic sea water, shrink when returned to normal sea water, but not at all to the same extent. The white half loses a great deal of water, the red half very little (Photographs 13, 14). The two halves become of approximately the same size as the two halves obtained from a normal egg centrifuged in normal sea water (Photographs 8, 9). The loss of water from the white halves takes place exceedingly rapidly. Lucké and co-workers (1927; 1931b; 1932a) have called attention to the much more rapid shrinking than swelling in the case of whole eggs.

When whole normal eggs are shrunken in hypertonic sea water, and returned to normal sea water, they likewise regain their normal size. When eggs are centrifuged in a hypertonic solution (125 per cent), the two halves, as stated above, are of nearly equal size, the red half being in most batches a little larger than the white half (Photograph 12). When these halves are returned to normal sea water, they gain water in approximately the same amount and at the same rate, so that they both become slightly larger, but keep approximately the same size relative to each other (Photographs 15, 16, 17). The white halves never attain the size of the white halves centrifuged off in normal sea water. If the halves from the hypertonic sea water are placed in hypotonic sea water (60 per cent), they still swell approximately the same amount, the red halves being in the batch pictured a little larger than the whites (Photograph 18).

Size of nuclei in hypo- and hypertonic sea water and their recovery in sea water (Table I and Plate I)

Though not directly related to the problem under discussion, the size of the mature nucleus in hypo- and hypertonic sea water is of sufficient interest to be recorded here. The nucleus of a normal mature unfertilized Arbacia egg is difficult to measure because it is imbedded in granules. However, when the egg is centrifuged, the nucleus lies in the clear layer under the oil cap and can easily be observed and measured in both the whole egg and the white half. The increase in size in hypotonic sea water, and the decrease in hypertonic sea water is quite apparent in photographs (Plate I). The nucleus of the normal egg in sea water measures approximately 11.5 μ in diameter; in 60 per cent sea water the diameter is 16 μ, an increase to two and a half times its volume; in 80 per cent sea water the diameter is 12.8 μ; in 125 per cent sea water the diameter is 9.6 μ, a decrease to about one half the volume of the normal nucleus (Table I A). The cell increases to about one and one half its volume in 60 per cent, and decreases to three quarters its volume in 125 per cent sea water. The percentage increase and decrease in volume of the nucleus is greater than the percentage increase and decrease in

---

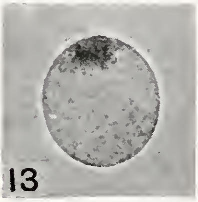
PLATE II

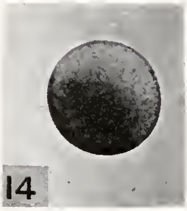
Photographs 13, 14. The two half-eggs obtained from centrifuging in hypotonic sea water (60 per cent, as shown in Photographs 2, 3) after their return to normal sea water.

Photographs 15, 16, 17. The two half-eggs obtained from centrifuging in hypertonic sea water (125 per cent, as shown in Photograph 12) after their return to normal sea water; the same halves at 15-minute intervals. There was no further change in size in photographs taken several hours later.

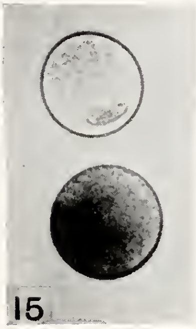
Photograph 18. The two half-eggs obtained from centrifuging in hypertonic sea water (125 per cent, as shown in Photograph 12), after placing them in 60 per cent sea water.

Same magnification as in Plate I, approximately 275 ×.

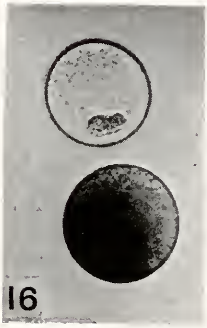

13


14

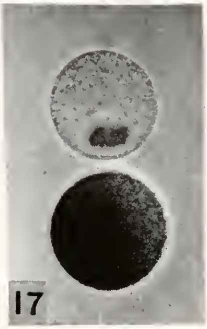
60% - 100%


15

12.30 p.m.

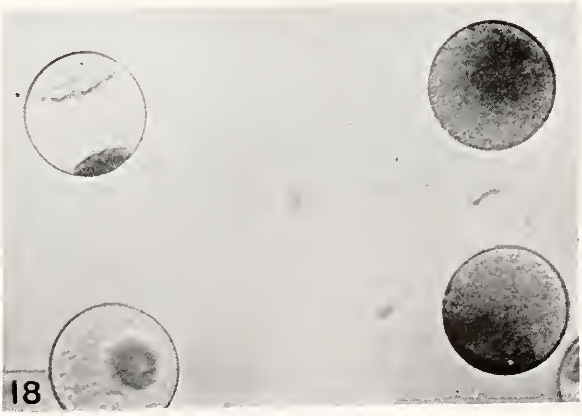

16

12.45 p.m.


17

1.p.m.

125% - 100%


18

125% - 60%

PLATE II

volume of the egg. This may be due to changes in metabolism, or to a smaller amount of osmotically inactive material in the nucleus, or it may be due to a difference in the nuclear and cell membranes. Skowron and Skowron (1926)[3] have noted a similar volume difference of the germinal vesicle of the *Sphaerechinus granularis* egg in comparison with the egg itself when treated with hypotonic solutions. And Beck and Shapiro (1936) found the same thing true for the germinal vesicle of the starfish egg swollen in 80 per cent sea water.

The nuclei of the whole Arbacia eggs returned to normal sea water after hypo- and hypertonic sea water, regain their normal size (Table I B), but at a much slower rate than the egg itself.

In the case of the germinal vesicle of the immature Arbacia egg, Churney (1942) concluded that it swells and shrinks reversibly in anisotonic solutions, and acts as a better osmometer than the egg itself. Beck and Shapiro (1936) have likewise found that the germinal vesicle of the starfish egg shrinks and swells in the same sense as the cell, and they have called attention to the fact that the rate is slower for the nucleus than for the egg to attain equilibrium. The mature nucleus of the Arbacia egg, therefore, seems in all respects similar to the immature nucleus (germinal vesicle) of Arbacia and other sea urchins, and of the starfish, with regard to swelling and shrinking. This is of interest and not necessarily to be expected because (1) the membrane of the mature nucleus is a new formation after the polar bodies are given off and (2) the contents of the mature and immature nucleus are different both in morphological structure (e.g. the nucleolus) and in the amount of material present; the volume of the germinal vesicle of Arbacia is about 50 times that of the mature nucleus.

Size of half-eggs obtained by centrifuging in normal sea water and then placing them in hypo- and hypertonic sea water (Table I C)

The swelling of half-eggs obtained by centrifuging eggs in a 100 per cent sea water-sugar medium and then placing them in hypotonic sea water has been adequately studied by Lucké (1932b, 1940). He found that both the half-eggs swelled in hypotonic sea water, but that the white half swelled a little more than the red half because the latter contained more of the osmotically inactive material, which he estimates as 12 per cent. His mean values are given in Table I D. My figures agree fairly well with his. In Table I C, my figures are given for the swelling and shrinking of the same eggs and half eggs as used in the other parts of the same experiment (Table I A and B). One may thus compare, in the same batch of eggs, the allocation of excess water in the two halves obtained by centrifuging before and after treating with hypotonic sea water; and similarly for the extraction of water in hypertonic sea water.

Discussion

With a constant centrifugal force of 10,000 × g, Arbacia eggs break less rapidly in hypotonic sea water and more rapidly in hypertonic sea water than they do in normal sea water. The tension at the surface is increased with the increase of surface area (Cole, 1932), so that if this factor alone were considered, the eggs should break less rapidly in hypotonic sea water, as they do. However, the densities of the half-eggs in comparison with the medium must also be considered, and these densities were not measured.

---

[3] These authors found no decrease in size of the germinal vesicle in hypertonic glucose ($\Delta = 2.57$), though the cell shrank 57 per cent; this seems strange in view of their results for hypotonic glucose.

With regard to the size of the two halves, it is seen from the data presented that when Arbacia eggs are kept in hypotonic sea water and centrifuged in a similar medium, the egg breaks so that the light half is much larger than the heavy half, whereas in normal sea water it is only slightly larger. The excess water is distributed largely to the light half. Conversely, when the eggs are kept in hypertonic sea water and centrifuged in a similar medium the egg breaks so that the light half is usually slightly smaller than the heavy half. Much of the water is taken away from the light half. This is perhaps what is to be expected since it is the clear layer in the light half that contains most of the osmotically active material. Similarly, the large white halves from the hypotonic sea water lose much more water when returned to normal sea water than do the smaller, more granular red halves. On the other hand, when the eggs are centrifuged in hypertonic sea water, the granules are more evenly distributed between the two halves, now nearly equal in size. The clear layer is small, the white half is quite granular, and the granules in the heavy half are not well packed, so that there is probably more liquid (osmotically active) material present among these granules than is apparent to the eye. Thus, when these two halves are returned to normal sea water, they swell approximately the same amount.

It might be of interest to compare the results obtained with hypo- and hypertonic sea water with those previously obtained by changing the centrifugal force (1941; compare Plate I of the present paper with Plate I of the previous paper). A low force acts similarly to hypotonic sea water; the heavy granules are well segregated, the light half is much larger than the heavy half, and the egg breaks apart less readily. A high force acts similarly to hypertonic sea water; the heavy granules are not well segregated, the light half is smaller than the heavy half, and the egg breaks apart more readily. Perhaps one might conclude that when the heavy granules are well packed, whether by using a low force or by adding water, the granular half is smaller in comparison with the less granular, and the egg breaks more slowly.

SUMMARY

1. *Arbacia punctulata* eggs, when centrifuged with a force of 10,000 × g, break less readily in hypotonic sea water, and more readily in hypertonic sea water than in normal sea water.

2. When broken apart in hypotonic sea water, the white half is very much larger than the red half. The white half is much larger than the white half obtained by centrifuging in normal sea water, the red half only slightly larger than the red half obtained in normal sea water.

3. When broken apart in hypertonic sea water, the white and red halves are of almost equal size, the white half usually a little smaller than the red half. When broken apart in normal sea water, the white half is somewhat larger than the red half. The white half from the hypertonic sea water is much smaller than the white half from normal sea water, the red half nearly the same size.

4. When the halves obtained by centrifuging in hypotonic sea water are returned to normal sea water, they both lose water, but the white half to a much greater extent than the red half. They become of approximately the same size as though they had been centrifuged in normal sea water.

5. When the halves obtained by centrifuging in hypertonic sea water are returned to normal sea water, they both take up water to about the same extent. The white half remains considerably smaller than when centrifuged in normal sea water.

6. The nucleus of the mature unfertilized egg increases perceptibly in hypotonic sea water and decreases in hypertonic sea water, to a greater percentage volume than the egg itself. It attains normal size on return to sea water.

LITERATURE CITED

Beck, L. V., and Shapiro, 1936. Permeability of germinal vesicle of the starfish egg to water. *Proc. Soc. Exp. Biol. and Med.*, **34**: 170–172.

Churney, L., 1942. Osmotic properties of the nucleus. *Biol. Bull.*, **82**: 52–67.

Cole, K. S., 1932. Surface forces of the Arbacia egg. *Jour. Cell. and Comp. Physiol.*, **1**: 1–9.

Harvey, E. B., 1932. The development of half and quarter eggs of Arbacia punctulata and of strongly centrifuged whole eggs. *Biol. Bull.*, **62**: 155–167.

Harvey, E. B., 1936. Parthenogenetic merogony or cleavage without nuclei in Arbacia punctulata. *Biol. Bull.*, **71**: 101–121.

Harvey, E. B., 1940. A comparison of the development of nucleate and non-nucleate eggs of Arbacia punctulata. *Biol. Bull.*, **79**: 166–187.

Harvey, E. B., 1941. Relation of the size of "halves" of the Arbacia punctulata egg to centrifugal force. *Biol. Bull.*, **80**: 354–362.

Harvey, E. N., 1931. The tension at the surface of marine eggs, especially those of the sea urchin, Arbacia. *Biol. Bull.*, **61**: 273–279.

Lucké, B. and co-workers.

  McCutcheon, M., and B. Lucké, 1927. The kinetics of exosmosis of water from living cells. *Jour. Gen. Physiol.*, **10**: 659–664.

  McCutcheon, M., B. Lucké, and H. K. Hartline, 1931a. The osmotic properties of living cells (eggs of Arbacia punctulata). *Jour. Gen. Physiol.*, **14**: 393–403.

  Lucké, B., H. K. Hartline, and M. McCutcheon, 1931b. Further studies on the kinetics of osmosis in living cells. *Jour. Gen. Physiol.*, **14**: 405–419.

  Lucké, B., and M. McCutcheon, 1932a. The living cell as an osmotic system and its permeability to water. *Physiol. Rev.*, **12**: 68–139.

  Lucké, B., 1932b. On osmotic behavior of living cell fragments. *Jour. Cell. and Comp. Physiol.*, **2**: 193–199.

  Lucké, B., 1940. The living cell as an osmotic system and its permeability to water. *Cold Spring Harbor Symposia*, **8**: 123–132.

Skowron, S., and H. Skowron, 1926. Les changements du rapport plasmonucléaire dans des oeufs pas mûrs d'Oursins sous l'influence de différences de la pression osmotique du milieu. *Bull. de l'Acad. Polonaise des Sc. et des Lettr. Ser. B.*, 1926: 859–879.

THE SPERMATOZOON AND FERTILIZATION MEMBRANE OF ARBACIA PUNCTULATA AS SHOWN BY THE ELECTRON MICROSCOPE[1]

ETHEL BROWNE HARVEY AND THOMAS F. ANDERSON[2]

(*The Marine Biological Laboratory, Woods Hole; the Biological Laboratory, Princeton University; and the Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania*)

Spermatozoa have been studied for many years with the light microscope, and the general structure of many kinds of spermatozoa has been described. This study of the spermatozoa of *Arbacia punctulata* was undertaken to throw further light on their structure by the use of the electron microscope.

The fertilization membrane of the Arbacia egg which is thrown off two minutes after fertilization is now generally believed to have been, at least in part, the plasma (or cell) membrane before fertilization. The fertilization membrane was therefore studied in the hope that the electron microscope would throw some light on the structure of the plasma membrane.

TECHNIQUE

The preparation of various kinds of biological material for the electron microscope has already been described in some detail (see Anderson, 1942, and references given therein). Briefly the procedure involves: (1) the complete removal of the sea water by washing several times with distilled water, in order to avoid the formation of salt crystals; (2) placing the material on a thin collodion membrane across a fine mesh wire screen (200 mesh per inch); (3) allowing it to dry; and (4) placing the screen in the electron microscope. In the present work an "RCA type B" microscope was used and the micrographs were taken with 60 kilovolt electrons.

The Arbacia sperm were taken directly from the testis of a freshly opened animal and diluted in sea water. They were then mounted on the collodion membrane to which they adhered, washed in several changes of distilled water, and then dried.

The preparation of the fertilization membranes presented greater technical difficulties; since they seemed to show no tendency to adhere to the collodion membranes, they had to be freed from the eggs and washed before they could be placed on the specimen screens. The fertilization membranes are formed about two minutes after fertilization of the eggs in sea water at 23° C. It was found that if the eggs are placed in distilled water one minute after formation of the fertilization membranes, these rupture and the egg contents flow out, leaving the empty membranes. If placed in distilled water a minute or two later, only part of the contents come out, and still later none at all. The procedure of washing the eggs several times in distilled water three minutes after fertilization was therefore adopted for separating the membranes from egg material and freeing them from salt. Under these conditions, the empty fertilization membranes sometimes retain their spherical shape, but usually collapse and become crinkled; they settle more slowly than the egg material to form a layer just above the bottom of the dish where the eggs lie. With a micropipette, under a binocular dissecting microscope, a number of membranes were taken up and deposited in tiny drops at the centers of the collodion membranes. The specimens were then dried in air and studied in the electron microscope.

---

[1] We are indebted to the RCA for the use of their electron microscope at Woods Hole during the summer of 1942.

[2] Formerly RCA Fellow of the National Research Council.

RESULTS AND DISCUSSION

Arbacia spermatozoon

The Arbacia spermatozoon at high magnification with the light microscope (Fig. 1) is observed to possess a pointed head with a flattened base adjacent to a short, slightly narrower, middle piece which seems to contain a pair of spherical bodies. The long thin filamentous tail extends from the middle piece. The head (with middle piece) measures approximately 4 μ long and 2 μ across the base; the tail is approximately 45 μ long. When placed in distilled water, the heads were observed to swell to about twice their original size.

In the electron microscope, the changes in structure caused by washing and drying are immediately apparent (Figs. 2 to 4). In most cases (except Figure 3), the heads have lost their characteristic arrow-head shape, and material appears to be flowing out of them. There is no distinct middle piece. The tails are, in most cases, coiled around the heads and consist of strands; the ends resemble frayed ends of rope unwrapped into separate strands (Fig. 2). The strands themselves are frequently detached, broken up, and strewn about the field (Fig. 3).

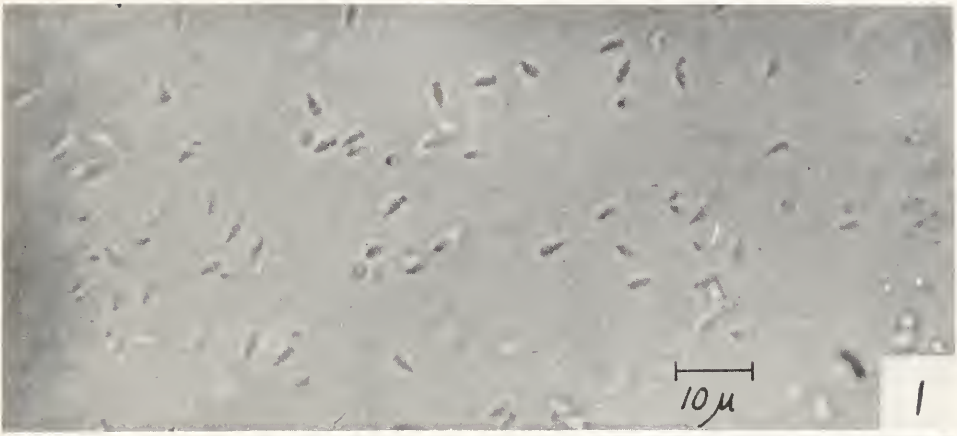
When examined more closely under higher magnification (Figs. 3, 4), a number of interesting features are apparent in the tails. Each tail appears to have been made up of about ten strands of uniform thickness, each having a diameter of about 50 mμ. In some of the micrographs, the tail has the appearance of a thick core surrounded by a sheath, but this appearance might be produced by a number of fibrils being superimposed at the center and flanked by one or two single fibrils. Occasionally one sees individual fibrils apparently broken up into short rods lined up in a row (Fig. 3), but this may be an artifact produced by drying, shrinking, and breaking. The regularly spaced cross striations which appear along the tail in certain areas (Fig. 4) may be characteristic of the material as has been reported for collagen fibers (Schmitt, Hall and Jakus, 1942) or may be an artifact of drying analogous to the formation of the rods noted above, but on a smaller scale.

PLATE I

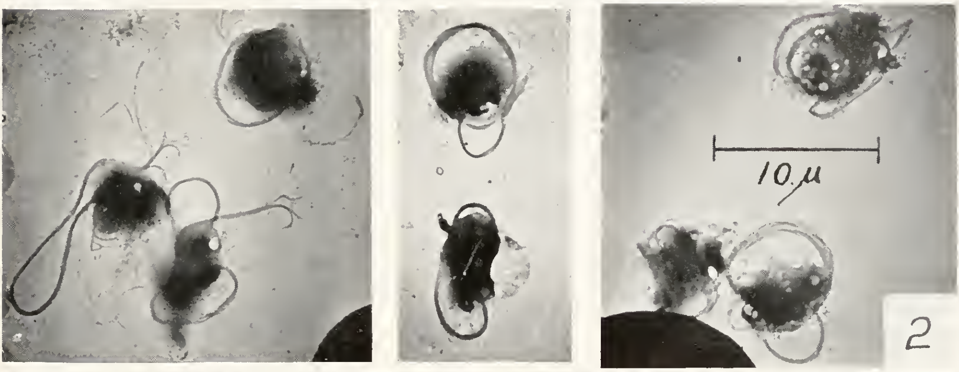
FIGURE 1. Living spermatozoa of *Arbacia punctulata* as photographed with the light microscope. × 1,000.

FIGURE 2. Spermatozoa of *Arbacia punctulata* micrographed with the electron microscope showing the appearance after washing in distilled water and drying. × 2,200.

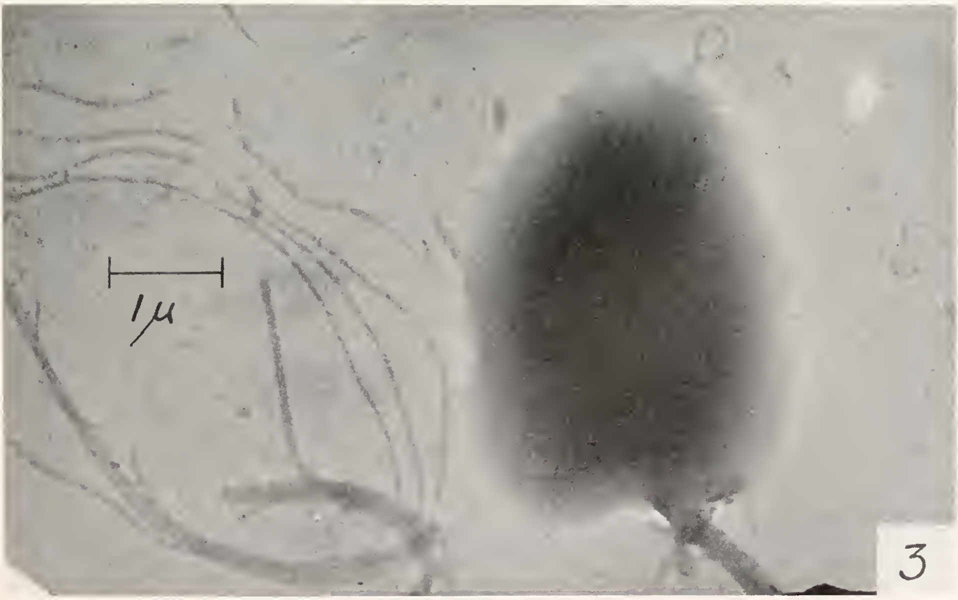
FIGURE 3. Head and fragments of the tail of a spermatozoon at high magnification with the electron microscope. × 15,000.



1



2



3

PLATE I

Unfortunately, the heads are too thick to show much internal structure. In some of the micrographs, one sees a small round area of low density which might represent a vacuole. There is also, in one of the micrographs (Fig. 4), a lighter area of the head having the appearance of a membrane. This is interpreted as the outer membrane left more or less intact on drying while the material inside has withdrawn and flowed out at the sides. It is not possible to determine the structure of the nuclear material from these micrographs. Some of the material found in the neighborhood of the heads appears to have interesting structure, such as the small rings, but it is impossible to identify it at this time.

Fertilization membrane of Arbacia

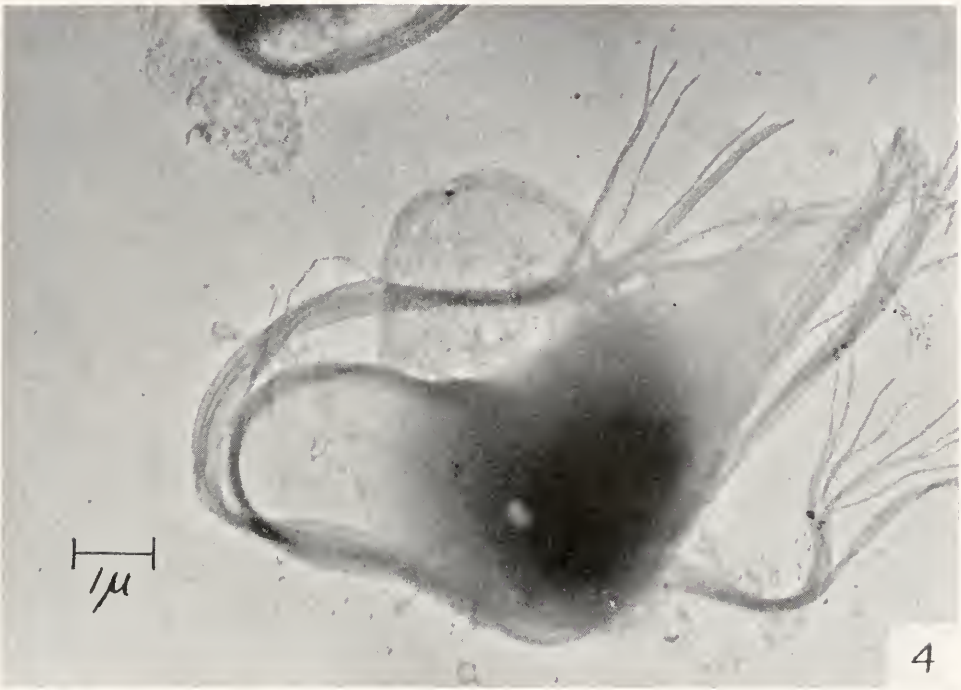
In the light microscope, the fertilization membrane of *Arbacia punctulata* appears as a uniformly thin and transparent membrane 3 to 5 μ from the surface of the egg. It is quite elastic when first formed, as shown by the fact that in high centrifugal fields it stretches from a sphere having a diameter of 80 μ to a spheroid having a length of 140 μ (Harvey, 1933, and unpublished observations). Five minutes after fertilization, however, the membrane thickens and hardens and resists stretching. Membranes freed from the eggs one minute after fertilization in distilled water have been observed to last 12 hours without any apparent change.

A number of electron micrographs of various fertilization membranes were taken and none showed anything but a thin amorphous structure (the membrane) sprinkled with what appears to be débris (Fig. 5). This débris may actually represent the structure of certain components of the cell or plasma membrane of the unfertilized egg, but the fact that they are neither characteristic in shape nor distributed in definite patterns on the surface prohibits one from attaching any special significance to them. There are no pores of sufficient size to be recognizable as such in the micrographs. From the apparent density of the micrograph one can estimate the thickness of the fertilization membrane, when first formed and dried, to be of the order of 25 mμ. It is of interest to note that this estimate is approximately the same as that of the membrane of the red blood cell. In the recent work of Zwickau (1941), who studied the red cell membranes with an electron microscope, the thickness of the membrane of the dried ghost is given as 20–30 mμ. Other estimates of the thickness of the intact red blood cell membrane, including water and diffusible proteins range from 20 mμ to as much as 50 mμ (see Ponder, 1942). The electron micrographs of the red blood cell membranes given by Zwickau show no definite structures.

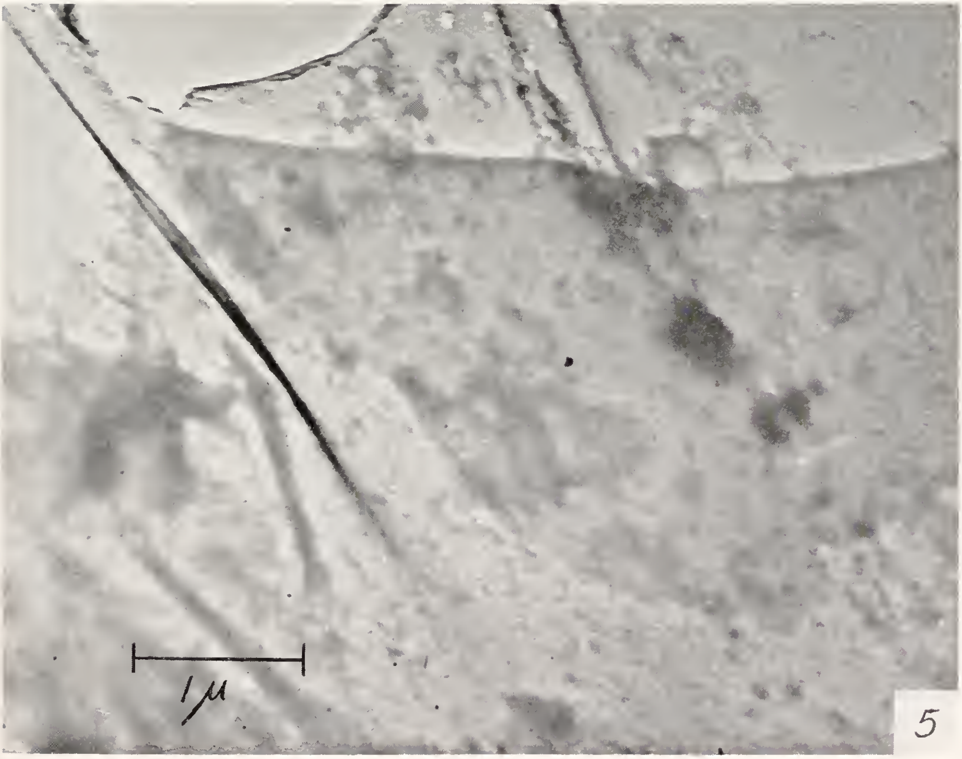
PLATE II

FIGURE 4. Spermatozoon, disrupted by distilled water, showing the multiple stranded structure of the tail with cross striations, and the remains of what may have been the membrane of the head—with the electron microscope. × 10,000.

FIGURE 5. Electron micrograph of the fertilization membrane of an *Arbacia punctulata* egg. At the top of the field is the collodion film on which the specimen is mounted with a hole in it at the upper left hand corner. The fertilization membrane comes up from the bottom of the field and folds over on itself near the top. The dark line extending from the upper left hand corner is a wrinkle in the film. Note the frayed edge of the fertilization membrane to the left of the middle of this wrinkle. × 22,000.



4



5

PLATE II

SUMMARY

1. As studied with the electron microscope, the tail of the *Arbacia punctulata* spermatozoon is found to disrupt into about ten distinct fibrils when it is washed in distilled water and dried. Each fibril is about 50 mμ in thickness. Regularly spaced cross striations also appear in the tail structure, but these may be produced in the washing and drying process.

2. A method of obtaining the fertilization membranes of *Arbacia punctulata* eggs free from egg material is described. When these were washed in distilled water and dried for examination, the electron microscope revealed no regular structures nor definite patterns. The thickness of the fertilization membrane, when first formed and dried is estimated to be of the order of 25 mμ.

LITERATURE CITED

ANDERSON, T. F., 1942. The application of the electron microscope to biology. *The Collecting Net*, **17**: 4–6.

HARVEY, E. B., 1933. Effects of centrifugal force on fertilized eggs of Arbacia punctulata as observed with the centrifuge-microscope. *Biol. Bull.*, **65**: 389–396.

PONDER, E., 1942. Quantitative aspects of the disc-sphere transformation produced by lecithin. *Jour. Exp. Biol.*, **19**: 220–231.

SCHMITT, F. O., C. E. HALL, AND M. A. JAKUS, 1942. Electron microscope investigations of the structure of collagen. *Jour. Cell. and Comp. Physiol.*, **20**: 11–33.

ZWICKAU, R., 1941. *Zur Frage d. Erythrocytenmembrane.* Inaug. Diss. From the Lab. f. Übermicroscopie d. Siemens u. Halske, Berlin.

THE DEVELOPMENT OF AN ENZYME (TYROSINASE) IN THE PARTHENOGENETIC EGG OF THE GRASSHOPPER, MELANOPLUS DIFFERENTIALIS *

JOSEPH HALL BODINE AND THEODORE NEWTON TAHMISIAN

(*Zoological Laboratory, The State University of Iowa, Iowa City*)

Introduction

Parthenogenesis has offered opportunities for investigating problems in almost every phase of experimental biology. In the grasshopper (*Melanoplus differentialis*) parthenogenesis has been studied from the developmental and cytological aspects by King and Slifer (1934). Studies on protyrosinase formation and activation in the normal fertilized grasshopper egg have been carried out in some detail in this laboratory and it, therefore, becomes of interest to compare such results with those from parthenogenetic eggs.

Materials and Methods

Female grasshopper nymphs (*Melanoplus differentialis*) in the third instar were segregated and raised free of males. At maturity their eggs were collected daily and kept in filter paper on moist sand at 25° C. These eggs were prepared for experimentation in the following manner: Approximately 150 eggs of a known chronological age and temperature history were placed in 0.9 per cent NaCl solution and each egg was scraped free of its chorion at the posterior end in order to determine the presence of a cuticle. Only those eggs with a cuticle were chosen. These in turn were sterilized in 70 per cent ethyl alcohol for 10 minutes. Ten to 25 eggs were taken from this lot and dissected to determine the presence of an embryo as well as its morphological age (Slifer, 1932). Of those remaining 100 were taken and placed in a glass mortar, rinsed with redistilled water, and then triturated with 0.9 per cent NaCl. The triturate was diluted to 10 ml. and centrifuged at 1,500 times G. for 10 minutes. The lipoidal *A* layer and the shell fragments constituting the major portion of the *C* layer (Bodine and Allen, 1938) were discarded since practically no protyrosinase is present in them. The protyrosinase content of the *B* layer was determined manometrically.

Each vessel of the Warburg manometer contained 1 ml. of the enzyme extract, 0.5 ml. of Sorensen's phosphate buffer (0.2 M. in respect to the phosphate) at pH 6.8, 0.3 ml. of 1 per cent aerosol OT solution, 0.9 per cent NaCl solution, and 0.3 ml. of a 0.4 per cent solution of tyramine-HCl in the side bulb. Ten minutes after equilibration at 25° C. the substrate in the side bulb was decanted into the main chamber of the vessel and the first reading taken two minutes after mixing. The manometers were shaken at 120 oscillations per minute through an amplitude of 2 cm.

---

* Aided by grant from the Rockefeller Foundation for research in cellular physiology.

The morphological age of the embryos in the diapause eggs was determined by removing the chorion and noting the position of the eye pigment. At diapause the head of the embryo is at the posterior end of the egg. After diapause the head of the embryo faces the anterior end of the egg making the determination of the morphological stage relatively simple (Slifer, 1932). Only those eggs which, in diapause, had a cuticle and an embryo were chosen for experiments. Such selections were especially necessary for parthenogenetic eggs to insure reproducible results.

Five day old parthenogenetic eggs were divided into two groups; one was kept as control and the other was irradiated at 1,000 r (Ray, 1938; Bodine and Allen, 1941). Both groups were then kept at 25° C. Daily determinations of the protyrosinase content were made starting with the tenth day after irradiation.

Fertilized eggs collected in the usual manner were allowed to develop at 25° C. and on the tenth day divided into two groups. One group was placed at 0° C. and the other was kept at 25° C. to serve as control. A second group of eggs was divided into two groups on the fifteenth day of prediapause development at 25° C. One group was left as a control at 25° C. while the other was placed at 0° C. A third group was divided on the fifteenth day of prediapause development at 25° C. Those placed at 0° C. were further divided after the tenth day and one lot of these was then placed at 25° C. Daily determinations of the protyrosinase content of the eggs were made.

Prediapause and diapause fertilized eggs were divided into two groups. One lot from each of these groups was kept as a control at 25° C. The other groups were separately placed into glass bottles and sealed. The eggs in the sealed bottles were then subjected for one hour to − 78° C. with the aid of dry ice and ether. The protyrosinase content of these four groups was determined immediately after the experimentals were so treated. Thereafter they were all placed at 25° C. and daily determinations of the protyrosinase content of the cold treated and control eggs were made.

RESULTS

During prediapause from the day of laying until the fifteenth day of development at 25° C. parthenogenetic eggs contain no detectable protyrosinase (Fig. 1). Enzymogenesis begins on approximately the fifteenth day of development and the enzyme increases in amount until the twenty-fifth day. At diapause the protyrosinase content of the parthenogenetic egg remains constant at the level attained on the twenty-fifth day. Unlike parthenogenetic eggs the protyrosinase of the normal fertilized egg appears much earlier, namely on the eighth day of development (Fig. 1), (Bodine and Boell, 1935). It gradually increases in amount until at diapause the protyrosinase content is at a maximum (Bodine and Allen, 1941). It is obvious that there is a marked lag in the appearance of protyrosinase in the parthenogenetic egg. King and Slifer (1934) described a lag in the morphological, cytological and developmental aspects of the parthenogenetic grasshopper egg.

Ray (1938) observed that irradiation of normal fertilized eggs with 1,000 r on the fifth day of development destroyed the embryo but did not affect the formation of protyrosinase. Later it was noted (Bodine and Allen, 1941) that irradiation with 1,000 r on the first day of development destroyed both the embryo and the serosa cells and that no protyrosinase was formed. Irradiation with 1,000 r on the fifth day after laying, however, was without effect on the function of the serosa cells in their formation of the yellow cuticle, white cuticle, and protyrosinase. It became of some interest, therefore, to compare the effect of a similar dose of x-irradiation on five day old parthenogenetic eggs. Since all parthenogenetic eggs do not develop (King and Slifer, 1934) it was necessary to compare the number of eggs that formed cuticle several days after irradiation with non-irradiated control parthenogenetic eggs. In both cases approximately 70 per cent formed cuticles. The presence of cuticle is important as an index of the functional state of the serosa which also seems to produce most of the protyrosinase (Bodine and Allen, 1941). In parthenogenetic eggs the formation of the cuticle begins approximately on the tenth day of development. The protyrosinase content between the fifteenth and twenty-fifth day of development was similar in irradiated and non-irradiated parthenogenetic eggs (Fig. 1). It should be pointed out that in this case also the amount of protyrosinase at diapause is half as much as in the normal fertilized irradiated eggs (Ray, 1938; Bodine and Allen, 1941).

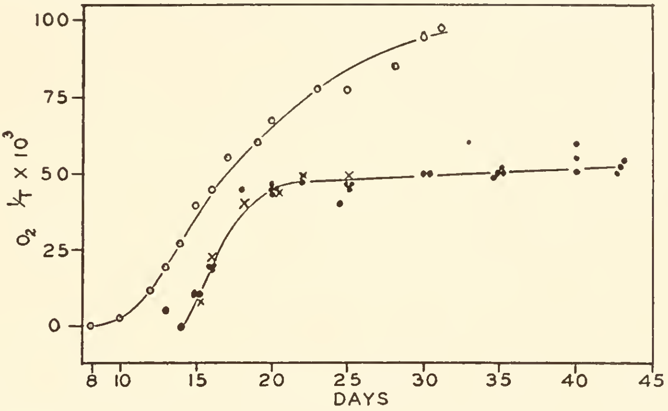


FIGURE 1. Shows amounts of enzyme in normal fertilized eggs and parthenogenetic eggs. Abscissae, developmental time in days at 25° C. Ordinate, reciprocal half oxidation period × 10^3. ○ = normal fertilized eggs. ● = parthenogenetic eggs. X = irradiated parthenogenetic eggs.

In order to check certain factors such as lag in development, high mortality, etc., possibly related to the formation of a reduced amount of enzyme in the parthenogenetic egg, the following experiments were carried out with fertilized eggs.

Fertilized eggs placed at 0° C. immediately after laying did not develop. Over a period of eighteen days no protyrosinase was detectable. When eggs so treated were placed at 25° C. protyrosinase appeared on the twenty-fifth day after laying. It should be pointed out that these eggs in reality were at a developmental temperature for 7 days ± 1 day (Fig. 2). The amount of protyrosinase and the development of the embryos were similar to control eggs kept for a similar period at developmental temperatures. Eggs placed at 0° C. on the fifteenth day of development also remained at this developmental stage. No change in the protyrosinase content over that found on the fifteenth day was detectable. When these eggs were returned to 25° C. development and protyrosinase content increased in a normal fashion (Fig. 2). The ultimate protyrosinase content of these eggs was similar to that found in normal ones regardless of the stage or the length of time they were inhibited.

Normal fertilized diapause eggs killed by subjection to − 78° C. show a slight drop in enzyme content immediately after freezing (Bodine and Allen, 1941). Thereafter the amount of enzyme does not change significantly for a period of 18 days at 25° C. On the other hand the protyrosinase content of the normal prediapause fertilized egg remains constant for a period of 18 days after subjection to the low temperature (Fig. 3).

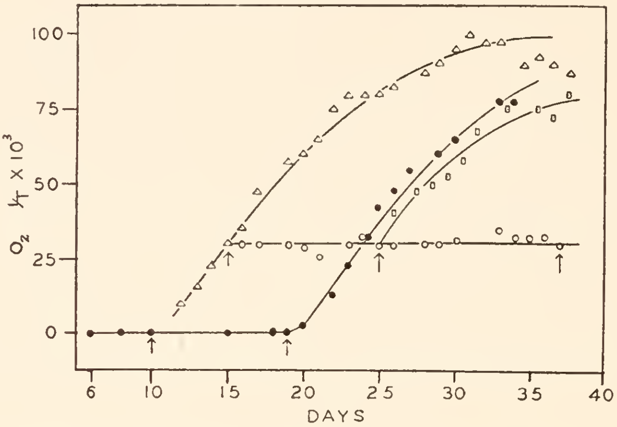


FIGURE 2. Shows effect of developmental block, due to low temperature, on the enzyme content of fertilized eggs. Ordinates as in Figure 1. Abscissae, time in days. Arrows indicate period of exposure to 0° C. △ = control eggs at 25° C. ● = put at 0° on tenth day; on eighteenth day put at 25° C. ○ = put at 0° C. on fifteenth day. □ = taken from 0° C. on the twenty-fifth day and put at 25° C.

DISCUSSION

In parthenogenetic eggs the lag in protyrosinase formation is doubtless due to the lag in the developmental aspects of these eggs (King and Slifer, 1934). The lesser amount of the protyrosinase ultimately produced in the parthenogenetic egg is a matter for some speculation.

In the course of parthenogenetic development, several anomalies occur, e.g., haploidy, retarded growth, undifferentiated growth of the embryonic cells, and a high rate of mortality (King and Slifer, 1934). Concerning a haploid condition, King and Slifer suggest that a *total* haploid condition in all probability does not permit the embryo to develop. On the other hand they have observed partial haploidy in individual embryos. They believe that in order for an embryo to develop certain of its cells essential for the propagation of the embryo, must become diploid while others may remain in a haploid condition. Haploid, diploid and triploid sets of chromosomes in individual embryos have been observed in the present work. Concerning the early differentiation of the serosa it may well be that these nuclei in the parthenogenetic egg originate from haploid cells. Since the serosa plays such an important part in the formation of protyrosinase (Bodine and Allen, 1941) it is probable that a haploid condition may produce half the amount of protyrosinase in comparison to the normal diploid egg. We were unable to observe chromosomes in the serosa nuclei after they were morphologically differentiated. Under normal conditions the serosa nuclei in fertilized eggs increase in size and chromatin content by a peculiar type of endomitosis (Tahmisian, Allen, and Bodine, 1942). This type of growth of the serosa nuclei was also observed in the parthenogenetic eggs. As far as we can determine, normal differentiated serosa cells in general do not deviate morphologically from those found in the parthenogenetic eggs. In one case only a serosa from a parthenogenetic egg had many small nuclei interposed with normal appearing large ones.

In order to ascertain the effect of retardation in the development of normal fertilized eggs on the formation of protyrosinase, several lots of eggs were retarded in development by subjection to 0° C. The results of these experiments have already been mentioned above (Fig. 2). No matter at what stage the eggs were experimentally retarded in relation to the time sequence of development, the maximum amount of enzyme ultimately formed is equivalent to the amount of protyrosinase found in the control eggs. We may, therefore, conclude that the reduced amount of protyrosinase in the parthenogenetic eggs can not be accounted for on the basis that at a previous time they had been retarded in their development. Since each of the parthenogenetic eggs selected contained an embryo and a cuticle the effect of undifferentiated growth may be ruled out.

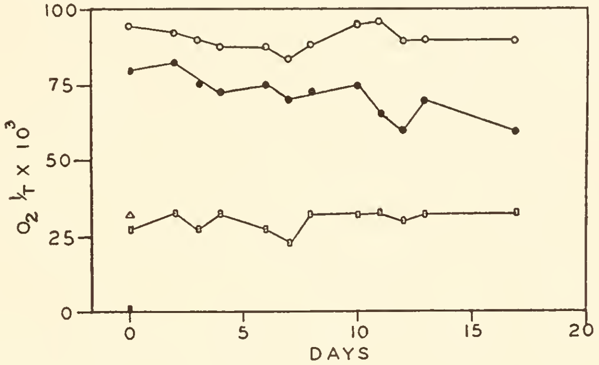


FIGURE 3. Shows effect of killing fertilized eggs by subjection to − 78° C. on their enzyme content. Ordinates and abscissae as in Figure 2. ○ = control diapause eggs. ● = diapause eggs subjected to − 78° C. △ = enzyme content of 15-day eggs. □ = 15-day eggs subjected to − 78° C.

King and Slifer (1934) also pointed out that many of the parthenogenetic eggs die during development. In spite of the fact that the parthenogenetic eggs were selected each day before analysis for protyrosinase it was possible that the results were due to the presence of eggs that apparently were normal but which really were dead. In order to determine the effect of killing the eggs on their protyrosinase content, two lots killed by subjection to − 78° C. were analyzed daily. It is apparent (Fig. 3) that no detectable change in enzyme content was noted in dead prediapause eggs for a period of 18 days. On the other hand there is a very small drop in the protyrosinase content of the killed diapause egg. As far as we can determine the parthenogenetic eggs used in all experiments were not dead. An egg, dead for 24 hours or more, changes color from a buff to dark sepia or black which in no case was observed in the parthenogenetic eggs used. We, therefore, infer that the protyrosinase content of the egg that has developed to a definite morphological age though dead contains the same amount of enzyme as a normal egg of the same age.

Another question which at present cannot be answered is suggested by the fact that all parthenogenetic eggs develop into females (King and Slifer, 1934). Is the protyrosinase content of normal fertilized female eggs one third that of the protyrosinase content of the males? If so, then a sample containing equal representation of eggs that will develop into males and females would of necessity have two times as much protyrosinase as do the eggs that will develop into females. And since all of the parthenogenetic eggs develop into females (King and Slifer, 1934), the presence of half the amount of protyrosinase in these eggs might, therefore, be accounted for on this basis.

The fact that the parthenogenetic eggs in practically all cases have exactly half as much protyrosinase as compared to the normal fertilized ones suggests that the male element donates some peculiar capacity to the normal developing egg to form twice as much protyrosinase as can be produced by the female element alone. Another possibility is that the unfertilized egg though appearing to be well coordinated for morphological differentiation is physiologically not well coordinated. It would be of interest to see if parthenogenetic eggs removed by another generation would contain still less protyrosinase.

Summary and Conclusions

1. Development and rate of growth of the enzyme tyrosinase have been studied in the parthenogenetic egg of the grasshopper, *Melanoplus differentialis*.

2. A marked lag in the appearance of the enzyme in the parthenogenetic egg occurs.

3. Total amount of enzyme found in the parthenogenetic egg is approximately 50 per cent of that found in the normal fertilized egg.

4. Parthenogenetic eggs subjected to x-irradiation on the fifth day of development show no change in the amount and rate of production of the enzyme—suggesting, as in normal eggs, the production of the enzyme by the serosa cells.

5. Results of experiments are presented which tend to show that arrested development, or killing of eggs, by low temperature do not produce lowered amounts of enzyme in eggs thus treated.

6. Possible explanations for the production by parthenogenetic eggs of lowered amounts of enzyme are given.

LITERATURE CITED

Bodine, J. H., and E. Boell, 1935. Enzymes in ontogenesis (Orthoptera). I. Tyrosinase. *Jour. Cell. and Comp. Physiol.*, 6: 263–275.

Bodine, J. H., and T. H. Allen, 1938. Enzymes in ontogenesis (Orthoptera). IV. Natural and artificial conditions governing the action of tyrosinase. *Jour. Cell. and Comp. Physiol.*, 11: 409–423.

BODINE, J. H., AND T. H. ALLEN, 1941. Enzymes in ontogenesis (Orthoptera). XX. The site of origin and the distribution of protyrosinase in the developing egg of a grasshopper. *Jour. Exp. Zool.*, **88**: 343–352.

KING, R. L., AND E. SLIFER, 1934. Insect development. Maturation and early development of unfertilized grasshopper eggs. *Jour. Morph.*, **56**: 603–619.

RAY, O. M., 1938. Effects of roentgen rays on the activation and production of the enzyme tyrosinase in the insect egg (Orthoptera). *Radiology*, **31**: 428–437.

SLIFER, E., 1932. Insect development. IV. External morphology of grasshopper embryos of known age and with a known temperature history. *Jour. Morph.*, **53**: 1–21.

TAHMISIAN, T. N., T. H. ALLEN, AND J. H. BODINE, 1942. Endomitosis (?) in grasshopper serosa cells. *Anat. Rec.* (Abstr.), **84**: 502–503.

A QUANTITATIVE STUDY OF ANAPHASE MOVEMENT IN THE APHID TAMALIA

HANS RIS

(*Department of Biology, The Johns Hopkins University, Baltimore, and the Marine Biological Laboratory, Woods Hole*)

No single phase of mitosis has been discussed as often as the anaphase movement of chromosomes. The precision of the movement, the relatively large distances covered and the possibility of correlation with definite cellular structures make it better suited for causal analysis than any other phase of cell division. A great number of ingenious hypotheses have been designed to account for the movement of chromosomes, making use of practically every known chemical and physical process which could bring chromosomes from the metaphase plate to the poles. But so far none has been satisfactory and none has been verified even partly by experiment. To some extent this failure is due to the difficulty of the subject. Another reason is the over-emphasis on deductive schemes which may explain a movement of bodies like chromosomes but which are without empirical foundation. This was clearly stated by Bělař (1929a) when he pointed out that we have to find out how the chromosomes move before we can ask what forces are responsible for this movement. What is needed then is a quantitative description of the chromosome movement derived from the study of living cells in division. There are in the literature only two such accounts: one by Bělař (1929a) in spermatocytes of the grasshopper (Chorthippus) and the other by Barber (1939) in Tradescantia staminal hair cells. Bělař derived his data from measurements on photographs which were taken at intervals of several minutes. This can give only a very rough picture of the chromosome movement. Barber measured the distance between disjoining kinetochores, again on photographs, at intervals of one-half or one minute and therefore could offer a more complete description of the anaphase movement. However, the position of the long chromosomes in the metaphase plate and in early anaphase make exact measurements in these stages almost impossible. The present investigation was undertaken to provide more data on the movement of chromosomes in living cells as a basis for both experimental attacks and theoretical interpretations.

MATERIAL AND METHODS

The bearberry aphid *Tamalia coweni* was found to be favorable material for the study of cell division in both spermatocytes and embryonic cells. Several males or parthenogenetic females are dissected in a drop of paraffin oil on a coverglass. The testes—or young embryos—come to lie in a small pool of body fluid surrounded by paraffin oil. The coverglass is then inverted over a depression slide. Cells have thus been kept alive and normally dividing for more than 10 hours. A glass container with ferrous ammonium sulphate between lamp and microscope prevented any heating due to the light source. The temperature varied from 22° to 26°. A good indication of the normality of conditions is given by the close agreement of the curves of different cells from different individuals (Fig. 1a and 4a). In addition spermatogonia and spermatocytes of *Protenor belfragii* and *Thelia bimaculata* were studied in a hanging drop of paraffin oil.

To analyze the movement of the chromosomes, a metaphase plate in side view is selected and with beginning anaphase the distance between the kinetochores of the daughter chromosomes recorded at intervals of one half to one minute with a camera lucida. This method was found to be simpler and more accurate than measurements on photographs. The error as determined from 20 measurements is ± 4 per cent. The various distances are then calculated in micra and plotted against time (Barber, 1939). We thus get a curve describing the movement of the chromosomes.

All forms studied here are characterized by a diffuse spindle attachment and therefore parallel disjunction. (Hughes-Schrader and Ris, 1941; Ris, 1942). This makes it easier to follow one single chromosome from metaphase to telophase. To avoid the error due to the curvature of the spindle a chromosome near the spindle axis is chosen. As a complement to the studies on live cells fixed and stained sections were used to measure the length of chromosomal fibers as well as the whole spindle with increasing separation of the daughter chromosomes.

The optics used consisted of a 2 mm. Zeiss oil immersion N.A. 1.4 and 15 × ocular.

Anaphase movement in secondary spermatocytes of Tamalia

The type of anaphase movement characteristic for the forms studied is most clearly shown in the secondary spermatocytes of Tamalia (Fig. 1a). When the daughter chromosomes begin to separate they are first connected by a "gray" mass which then breaks up into a few strands. These probably are identical with the Feulgen positive chromosome connections found in fixed cells (Ris, 1942). In a frontal or end view the chromosomes have a very characteristic dumb bell shape. The movement of the chromosomes is slow until all these connections have disappeared. Now it increases in speed and remains nearly uniform for several minutes, when it comes to a halt for about two minutes. The motion is then resumed only to slow down once more as the end of anaphase is approached. The second movement after the plateau in the curve coincides with the elongation of the cell. Previously the cell is spherical or in rare cases has elongated only slightly. Within about ten seconds after the beginning of elongation the cleavage furrow appears (arrow in Fig. 1a).

How can the interruption in the movement of the chromosomes be explained? The coincidence of cell elongation and the second movement of the chromosomes suggests that both may be connected with a stretching of the spindle. We could then picture the anaphase movement as composed of two phases: in the first the chromosomes approach the poles, or in other words, the chromosomal fibers shorten.[1] In the second phase the spindle stretches and moves the chromosomes farther apart. To prove this hypothesis we must take recourse to stained sections

---

[1] Since nothing is known about the mode of action of chromosomal fibers the term "shortening of chromosomal fibers" is used throughout this paper.

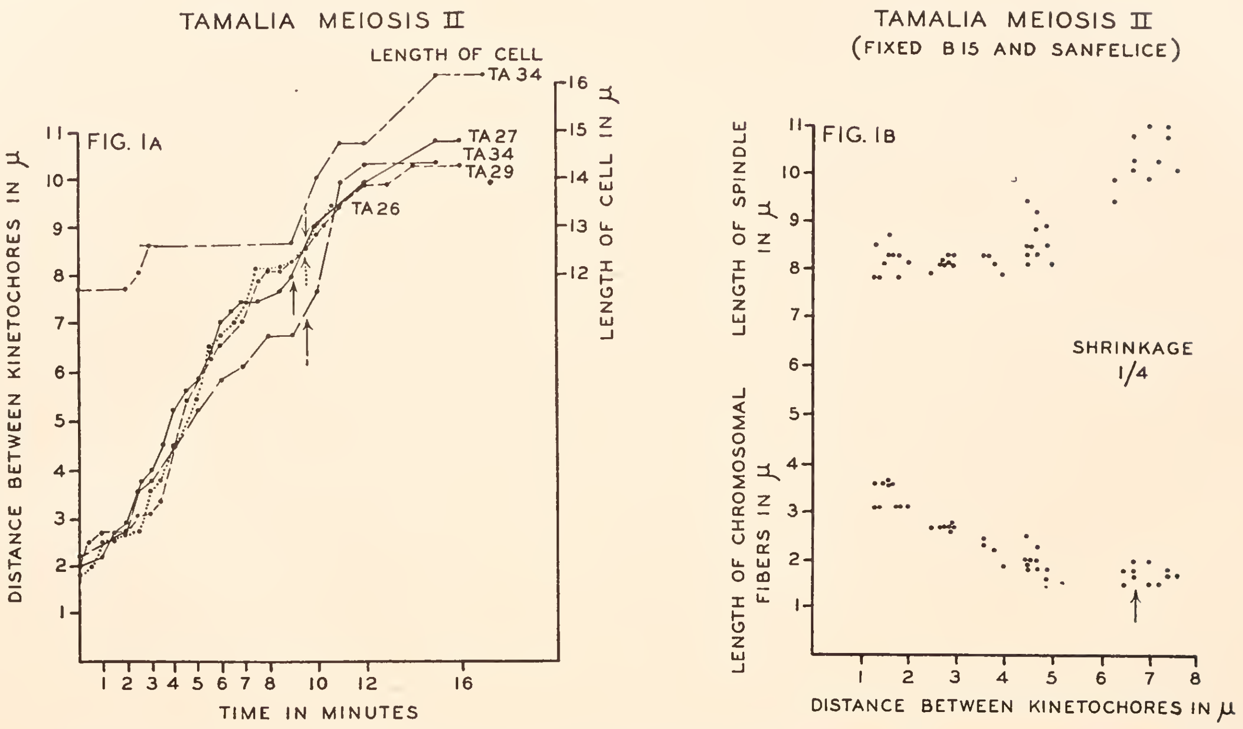


FIGURE 1a and 1b. Chromosome movement in secondary spermatocytes of Tamalia. 1a: measurements on living cells. The arrow marks the appearance of the cleavage furrow. For Ta 34 both distance between kinetochores and length of cell are plotted. 1b: measurements on fixed cells.

where we can measure the length of chromosomal fibers and spindle for various distances between the daughter chromosomes. Such measurements are plotted in Figure 1b. They show clearly that in the first part of the movement the chromosomal fibers shorten while the spindle remains constant in length. In the second phase the chromosomal fibers remain constant while the spindle begins to stretch, causing the further movement of the chromosomes. Making allowance

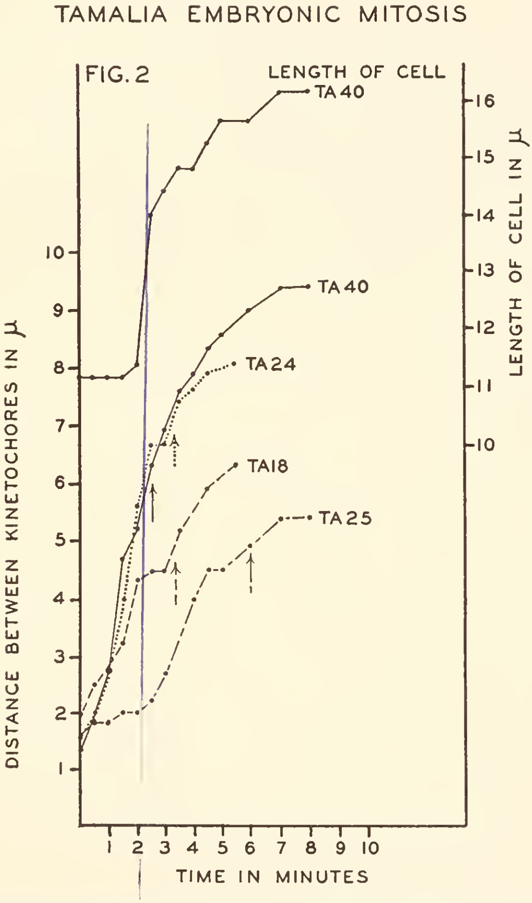


FIGURE 2. Chromosome movement in embryonic cells of Tamalia. Measurements on living cells. For Ta 40 both distance between kinetochores and length of cell are plotted.

for shrinkage at fixation, Figures 1a and 1b can be compared. Shrinkage was calculated by comparing the maximum separation of daughter chromosomes in living and fixed cells and results in a shortening of the interchromosomal distance by one-fourth. In the living cell the break in the curve occurs when the daughter chromosomes are from 7 to 8 μ apart, in the fixed cells accordingly at a separation of 5 to 6 μ. It is also interesting that the elongation of the cell corresponds closely to the increase of spindle length (increase in length of cell 4 μ, of fixed spindle 3 μ).

The movement of the chromosomes in this division can now be described in the following way: first slowly, then faster the chromosomes approach the poles apparently through the action of the chromosomal fibers. When they are from 7 to 8 μ apart this movement ceases and for a short time the chromosomes come to rest. Then the spindle begins to elongate, causing the final separation of the chromosomes. The distance from chromosomes to poles remains constant in this latter phase.

Anaphase movement in embryonic cells of Tamalia

Young embryos dissected from parthenogenetic females have many somatic cells in division. Curves for the anaphase movement are obtained as in spermatocytes. As there are many different types of cells of various sizes the curves differ quantitatively. The character of the movement, however, is the same in all cells and identical with that in secondary spermatocytes (Fig. 2). There is the initial slow movement, the first fast movement, the pause and the second movement coinciding with cell elongation. Because of the difference of the cells a comparison with measurements of fixed material is impossible. Yet the curves agree well enough with those of secondary spermatocytes to justify the conclusion that the nature of the movement is the same. The velocity of the chromosomes is greater than in spermatocytes and large enough so that the chromosomes can actually be seen in motion under the microscope.

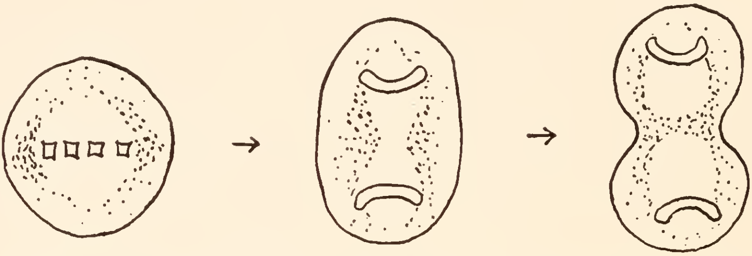


FIGURE 3. Anaphase in embryonic cell of Tamalia. Penetration of cytoplasmic granules in between the daughter plates. See text.

The observation of these cells during anaphase furnishes some interesting data on the spindle. The cytoplasm contains a great number of dark granules of various sizes. When the spindle is formed at metaphase they accumulate along its surface and thus outline its shape. In constant Brownian movement they can be seen bouncing off the surface of the spindle, but never penetrating it. Towards the end of metaphase the majority of granules has accumulated around the equatorial region of the spindle. In the first part of anaphase the spindle retains its characteristic shape, outlined by the cytoplasmic granules. As soon as the cell begins to elongate, indicating the stretching of the spindle, the granule-free region between the daughter plates becomes constricted in the middle and shaped like an hour glass. Soon afterwards cytoplasmic granules rush into the midregion of the spindle, continuously in unrestricted Brownian movement (Fig. 3).

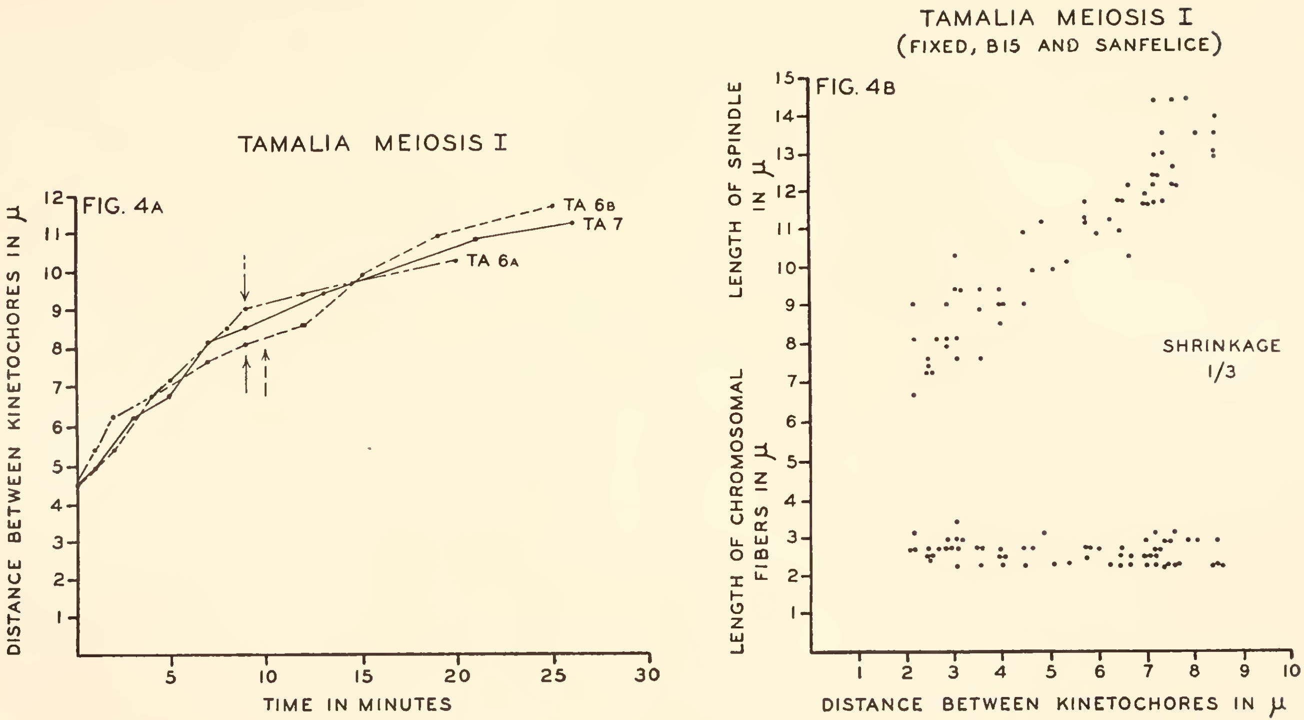


FIGURE 4a and 4b. Chromosome movement in primary spermatocytes of Tamalia. 4a: measurements on living cells. 4b: measurements on fixed cells.

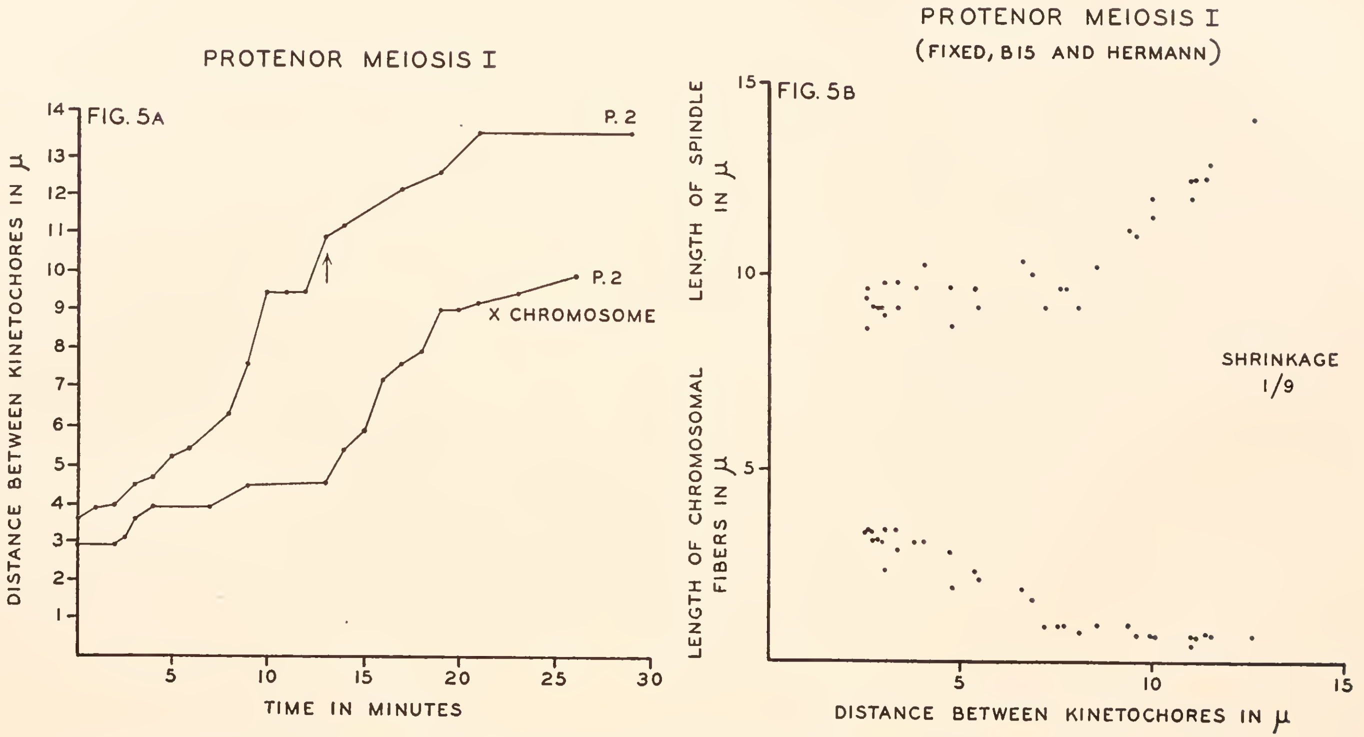


FIGURE 5a and 5b. Chromosome movement in primary spermatocytes of Protenor. 5a: measurements on a living cell, curve for autosomes and lagging X chromosome. 5b: measurements on fixed cells.

Anaphase movement in primary spermatocytes of Tamalia

The first spermatocyte division of the aphid is unusual in several ways. The univalent X chromosome is stretched into a flat sheet at anaphase and passes undivided into the larger of the unequal daughter cells (cf. Ris, 1942).

The anaphase movement also is different from that in cells previously described (Fig. 4a). The chromosomes very soon reach their maximum velocity and then gradually slow down towards the end of anaphase. The curve resembles the second movement in secondary spermatocytes, which was found to be caused by spindle elongation. Indeed the measurements of chromosomal fibers and spindle in fixed cells show that the entire movement of the chromosomes is due to the stretching of the spindle. The chromosomal fibers remain constant in length, i.e., the chromosomes do not get nearer the poles (Fig. 4b). The arrow in Figure 4a marks the appearance of the cleavage furrow.

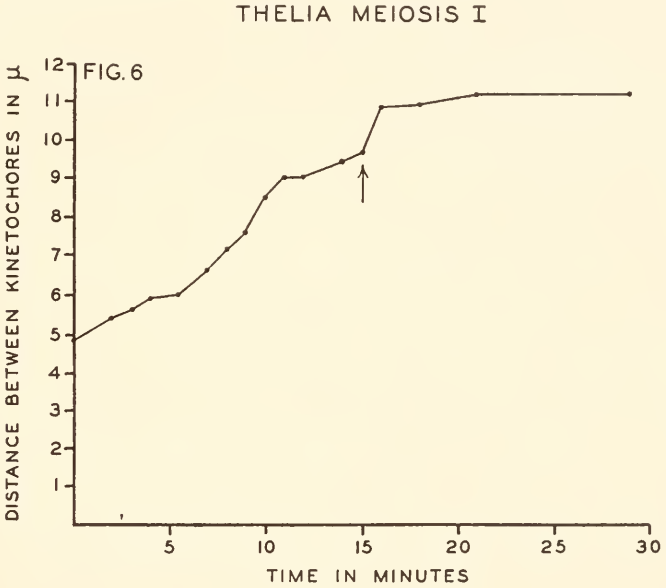


FIGURE 6. Chromosome movement in a primary spermatocyte of Thelia.

Is this kind of anaphase characteristic for primary spermatocytes or is it peculiar to the aphid? To answer this question the anaphase movement in primary spermatocytes of the hemipteran Protenor and the homopteran Thelia was analyzed.

Anaphase movement in primary spermatocytes of Protenor and Thelia

The chromosome movement in a primary spermatocyte of Protenor is shown in Figure 5a. The curve for the autosomes is of the same type as those found for somatic mitosis and secondary spermatocytes in the aphid. Again the cleavage furrow appears shortly after the second movement has started. Measurements of fixed cells finally show that anaphase here too consists of the two phases, the approach to the poles and the spindle elongation.

Interesting is the behavior of the univalent X chromosome. In the first meiotic division it splits equationally but the daughter chromosomes lag behind the autosomes (Schrader, 1935). What is the reason for this delay? The curve for the X chromosome in Figure 5a shows that it is the first part of anaphase which differs from that of the autosomes. Chromosomal fibers are present (Schrader, 1935), but if they are responsible for the movement towards the poles, they are in some way hampered in their function. In the second phase of the movement, which is related to the stretching of the spindle, the X chromosome behaves like the autosomes and even partially catches up with them.

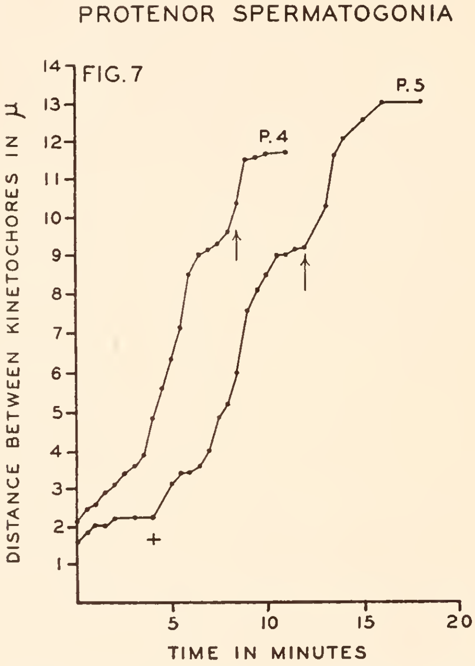


FIGURE 7. Chromosome movement in two spermatogonia of Protenor.

The first meiotic anaphase of Thelia is similar in character to that of Protenor (Fig. 6) and thus also of the same type as found in somatic cells and secondary spermatocytes of the aphid. It must be concluded, therefore, that the anaphase movement of the first meiotic division in Tamalia is different from that in Protenor and Thelia and represents an exceptional case.

Anaphase movement in spermatogonia of Protenor

In Figure 7 the anaphase movement in two spermatogonia of Protenor is recorded. In P_4 the distance between the ends, in P_5 that between the middle of two daughter chromosomes was measured. A comparison of the two curves shows how the ends of the chromosomes separate first while the midregion lags until the daughter chromosomes are fully separated (+ in P_5). Again the movement consists of two phases, separated by a short pause.

DISCUSSION

The measurements on living cells have furnished curves which describe in detail the movement of the chromosomes at anaphase. In the cells studied it was found to be composed of two parts. The first can be described as the shortening of the chromosomal fibers which moves the chromosomes towards the poles. The second consists of the elongation of the spindle, resulting in a further movement of the chromosomes.

TABLE I

Anaphase movement in secondary spermatocytes and embryonic cells of Tamalia.
d.k. = *distance between kinetochores;* l.c. = *length of cell*

	Spermatocytes II					Embryonic cells				
Time (minutes)	Ta 26 25° C. d.k.(μ)	Ta 27 24° C. d.k.(μ)	Ta 29 24° C. d.k.(μ)	Ta 34 23° C.		Ta 18 24° C. d.k.(μ)	Ta 24 26° C. d.k.(μ)	Ta 25 25° C. d.k.(μ)	Ta 40 22° C.	
				d.k.(μ)	l.c.(μ)				d.k.(μ)	l.c.(μ)
0	1.8	2.0	2.0	2.2	11.7	2.0	1.6	1.6	1.4	11.2
½	2.0	—	2.5	—	—	2.5	2.0	1.8	1.8	11.2
1	2.5	2.2	2.7	—	—	2.7	2.7	1.8	2.7	11.2
1½	2.5	2.7	2.7	—	—	3.2	4.0	2.0	4.7	11.2
2	2.7	2.9	2.7	2.7	11.7	4.3	5.6	2.0	5.2	11.4
2½	2.7	3.8	3.1	3.6	12.1	4.5	6.7	2.2	6.3	14.0
3	3.6	4.0	3.1	3.8	12.6	4.5	6.7	2.7	6.9	14.4
3½	3.8	4.5	3.3	—	—	5.2	7.4	—	7.6	14.8
4	4.5	5.2	4.5	4.5	12.6	—	7.6	4.0	7.9	14.8
4½	4.9	5.6	5.4	—	—	5.9	7.9	4.5	8.3	15.3
5	5.4	5.8	—	5.2	12.6	—	—	4.5	8.6	15.7
5½	6.5	6.3	6.3	—	—	6.3	8.1	—	—	—
6	6.7	7.0	6.5	5.8	12.6			4.9	9.0	15.7
6½	7.0	7.2	—	—	—			—	—	—
7	7.4	7.4	7.0	6.1	12.6			5.4	9.4	16.2
7½	8.1	7.4	7.9	—	—			—	—	—
8	—	—	8.1	6.7	12.6			5.4	9.4	16.2
8½	8.1	7.6	8.1	—	—					
9	—	7.9	8.3	6.7	12.6					
9½	8.5	8.5	8.5	—	—					
10	9.0	9.0	8.8	7.6	14.0					
10½	9.4	—	9.0	—	—					
11	—	—	9.4	9.9	14.9					
12		9.9	9.9	10.3	14.9					
13		—	9.9	10.3	16.2					
14		—	10.3	—	—					
15		10.8	—	10.3	16.2					
16		10.8	10.3							

In general, this picture of anaphase agrees with Bělař's hypothesis which resolves anaphase into (1) the action of the "Zugfaser" and (2) that of the "Stemmkörper." However, the chromosomal fibers, in the aphid at least, do not attach to a continuous fiber ("Leitfaser"), but form direct connections from the chromosome to the pole. No continuous fibers can be seen in this form. There is also little in favor of a specific differentiation of the region between the daughter-chromosomes into a "Stemmkörper." The intrusion of cytoplasmic granules into the equatorial region of the spindle (page 168) is evidence that this part of the spindle is not a rigid "Stemmkörper," but rather less viscous than the rest of the spindle. It is more likely that the spindle as a whole elongates, though probably to a greater extent in the equatorial region. Only actual measurements can clarify this point.

Table II

Anaphase movement in primary spermatocytes of Tamalia (Ta), *Protenor* (P), *and Thelia* (Th). d.k. = *distance between kinetochores*

Time (minutes)	Ta 6a 23° C. d.k.(μ)	Ta 6b 23° C. d.k.(μ)	Ta 7 25° d.k.(μ)	P 2 25° C.		Th 1 25° C. d.k.(μ)
				autosomes d.k.(μ)	X chromosome d.k.(μ)	
0	4.5	4.5	4.5	3.6	2.9	4.9
1	4.9	5.4	4.9	3.8	—	—
2	5.4	6.3	—	4.0	2.9	5.4
2½	—	—	—	—	3.1	—
3	—	—	6.3	4.5	3.6	5.6
4	6.7	6.7	—	4.7	3.8	5.8
5	—	7.2	6.7	5.2	—	—
5½	—	—	—	—	—	6.0
6	—	—	—	5.4	—	—
7	7.6	—	8.1	—	3 8	6.7
8	—	8.5	—	6.3	—	7.2
9	8.1	9.0	8.5	7.6	4.5	7.6
10	—	—	—	9.4	—	8.5
11	—	—	—	9.4	—	9.0
12	8.5	9.4	—	9.4	—	9.0
13	—	—	9.4	10.8	4.7	—
14	—	—	—	11.2	5.4	9.4
15	9.9	—	—	—	5.8	9.7
16	—	—	—	—	7.2	10.8
17	—	—	—	12.1	7.6	—
18	—	—	—	—	7.9	10.8
19	10.8	—	—	12.6	9.0	—
20	—	10.3	—	—	9.0	—
21	—		10.8	13.5	9.2	11.2
23	—		—	—	9.4	—
25	11.7		—	—	—	—
26			11.2	—	9.9	—
29				13.5		11.2

The shape of the chromosomes at anaphase indicates that the chromosomal fibers exert a pull on the kinetochore. This is not only seen when the chromosomal fibers shorten and bring the chromosomes to the poles, but also in the first spermatocyte of Tamalia where spindle elongation alone moves the chromosomes. The motion is therefore transmitted from the spindle to the chromosomes through the chromosomal fibers. The elongating spindle then does not push the chromosomes apart, but separates the poles. The chromosomal fibers, which in some way must be anchored to the polar regions then begin to pull at the spindle attachments of the chromosomes (cf. Ris, 1942; Fig. 84–90).

In the aphid, Protenor, and Thelia the two components of the anaphase movement are completely separated in time. How far can this type of movement be generalized? Barber (1939) in staminal hair cells of Tradescantia found simple S-shaped curves. He drew similar curves also through the points furnished by Bělař's photographs of anaphase in spermatocytes of the grasshopper (Chorthippus). Bělař's points are, however, so far apart that the lines drawn through them are purely hypothetical; they may or may not be simple. In Tradescantia staminal hair cells, as in other somatic plant cells, there is no elongation of the spindle and cell (cf. Bělař's photographs, 1929b). We may compare therefore this entire anaphase movement with the first part of that in the aphid. In both cases rather flat S-shaped curves are found. For the grasshopper preliminary measurements have shown that the chromosome movement differs from that of the aphid since the spindle begins to elongate before the shortening of the chromosomal fibers is completed.

TABLE III

Anaphase movement in spermatogonia of Protenor. d.k. = *distance between kinetochores*

Time (minutes)	P 4 25° C. d.k.(μ)	P 5 25° C. d.k.(μ)	Time (minutes)	P 4 25° C. d.k.(μ)	P 5 25° C. d.k.(μ)
0	2.2	1.6	8	9.7	5.2
½	2.5	1.8	8½	10.3	6.0
1	2.7	2.0	9	11.5	7.6
1½	2.9	2.0	9½	11.5	8.1
2	3.1	2.2	10	11.7	8.5
2½	3.4	—	10½	—	9.0
3	3.6	2.2	11	11.7	9.0
3½	3.8	—	11½		9.2
4	4.9	2.2	12		9.2
4½	5.6	—	13		10.3
5	6.3	3.1	13½		11.7
5½	7.2	3.4	14		12.1
6	8.5	3.4	15		12.6
6½	9.0	3.6	16		13.0
7	9.2	4.0	18		13.0
7½	9.4	4.9			

The anaphase curve with a distinct separation of the two components is found in three Hemiptera and Homoptera, but in no other form analyzed so far. One may therefore assume that it is related to the special kind of spindle apparatus found in these forms, namely, the diffuse spindle attachment. Should this be confirmed by further studies on other forms it would give additional evidence for the functional importance of structures like chromosomal fibers still believed by some investigators to be artifacts. It would also be an interesting example of how variations in cellular processes are related to differences in structure.

The behavior of the X chromosome in the first spermatocyte of Protenor is of great interest. Chromosomal fibers are present in metaphase and anaphase, but, as the analysis of the movement in a living cell shows, they are hindered in their normal functioning so that the X chromosome lags behind the autosomes on its way to the poles. This provides a mechanism for individual movements of chromosomes. A similar condition may be responsible for the lagging of specific chromosomes in elimination divisions of Sciara, Oligarces, etc.

The velocity of the chromosomes at anaphase is of great interest. The maximum velocities in the various divisions studied are brought together in table IV. The velocities due to the shortening of the chromosomal fibers and spindle elongation are recorded separately. The greatest velocity in embryonic cells of Tamalia is 2 μ per minute, or about 3 mm. in 24 hrs. As comparison the maximum velocity in Tradescantia staminal hair cells reported by Barber (1939) is added to the table.

TABLE IV

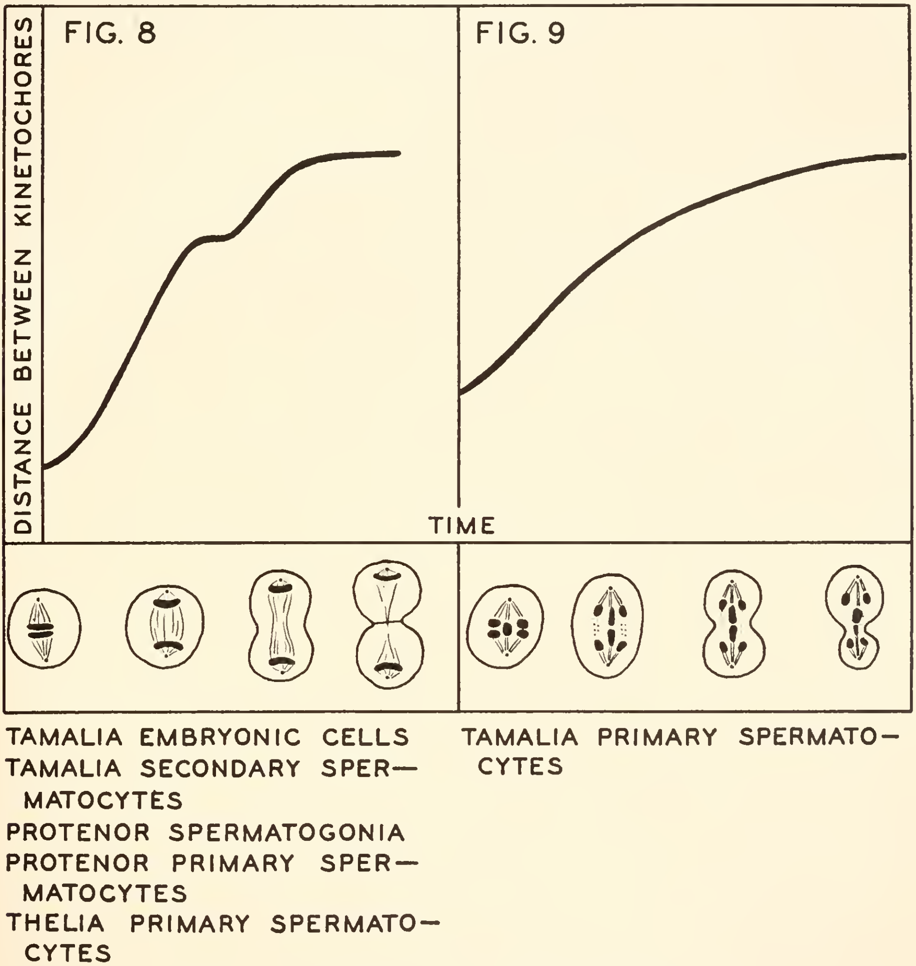
Maximum velocities of chromosomes. Micra/minutes. 23–26° C.

	Somatic mitosis		Spermatogonia		Meiosis I		Meiosis II	
	chromosomal fibers	spindle	chromosomal fibers	spindle	chromosomal fibers	spindle	chromosomal fibers	spindle
Tamalia	0.7–2	0.3–1.1			—	0.3	0.9–1.2	0.4–1.1
Protenor			1.3–1.6	0.3–0.5	0.9	0.7		
Thelia					0.4	0.5		
Tradescantia (Barber 1939)	1.2 (20° C.)							

CONCLUSIONS

The character of the chromosome movement at anaphase varies in different groups of organisms. It is possible to describe these differences as modifications in the behavior of components of the mitotic apparatus, such as chromosomal fibers and spindle body. Thus in Tradescantia staminal hair cells there is only the movement to the poles, in the first meiotic division of Tamalia only the elongation of the spindle (diagram Fig. 9). In regular divisions of Hemiptera and Homoptera the action of chromosomal fibers and spindle elongation are separated in time (diagram Fig. 8), in the grasshopper, however, they act simultaneously. These functional differences are correlated with variations in the spindle structure (diffuse against localized spindle attachment).

Measurements of chromosome movement such as those reported by Barber (1939) and in this paper represent a first step in the analysis of anaphase, namely a quantitative description of the processes observed in the cell. The movement must then be separated into its components and related to the cellular structures which are found to be essential for regular separation of chromosomes (kinetochore, chromosomal fibers, spindle, etc.). A theory of chromosome movement must be established first on a biological plane, accounting for the many modifications of anaphase as variations of these mitotic organelles. Finally an experimental analysis of the nature of these structures and the changes they undergo during mitosis can provide an empirical basis for a physico-chemical theory of mitotic movement.



Figures 8 and 9. Diagrams illustrating the chromosome movement in forms with diffuse spindle attachment. 8: the typical anaphase curve. 9: the exceptional curve in primary spermatocytes of Tamalia.

SUMMARY

1. The movement of chromosomes at anaphase was measured in living cells of Tamalia, Protenor and Thelia. The distance between the separating chromosomes plotted against time produces curves which describe accurately the chromosome movement. In embryonic cells and secondary spermatocytes of Tamalia, spermatogonia and primary spermatocytes of Protenor, and a primary spermatocyte of Thelia the curves consist of two S-shaped components separated by a plateau. The second part of the movement coincides with the elongation of the cell.

2. In stained sections the length of chromosomal fibers and the spindle was measured at various stages of chromosome separation. A comparison with the data from living cells shows that in the first part of anaphase the chromosomal fibers shorten, i.e., the chromosomes approach the poles. In the second part the spindle elongates and thus produces a further movement of the chromosomes.

3. The chromosome movement in the otherwise exceptional anaphase of primary spermatocytes in Tamalia is characterized by a simple unbroken curve. Measurements on stained cells demonstrate that the movement is due entirely to spindle elongation. The chromosomal fibers remain constant in length and the chromosomes therefore do not approach the poles.

4. Since the double curve was found in all Hemiptera and Homoptera studied but not in the grasshopper (unpublished results) this type of anaphase movement is probably related to the diffuse spindle attachment found in these insects. This points out the functional significance of structural variations.

5. The curves for the primary spermatocyte of Protenor show that the lagging of the daughter chromosomes of the univalent X chromosome is due to an abnormal first part of the movement. This indicates some impairment in the functioning of their chromosomal fibers. The exceptional behavior of a chromosome can thus be traced to one particular factor of the anaphase movement.

LITERATURE CITED

BARBER, H. N., 1939. The rate of movement of chromosomes on the spindle. *Chromosoma*, **1**: 33–50.

BĚLAŘ, K., 1929a. Beiträge zur Kausalanalyse der Mitose. II. Untersuchungen an den Spermatocyten von Chorthippus (Stenobothrus) lineatus Panz. *Roux' Arch. f. Entw. mech.*, **118**: 359–484.

BĚLAŘ, K., 1929b. Beiträge zur Kausalanalyse der Mitose. III. Untersuchungen an den Staubfadenhaarzellen und Blattmeristemzellen von Tradescantia virginica. *Z. Zellforsch.*, **10**: 73–134.

HUGHES-SCHRADER, S., AND H. RIS, 1941. The diffuse spindle attachment of coccids, verified by the mitotic behavior of induced chromosome fragments. *Jour. Exp. Zool.*, **87**: 429–456.

RIS, H., 1942. A cytological and experimental analysis of the meiotic behavior of the univalent X chromosome in the bearberry aphid Tamalia (= Phyllaphis) coweni (Ckll.). *Jour. Exp. Zool.*, **90**: 267–330.

SCHRADER, F., 1935. Notes on the mitotic behavior of long chromosomes. *Cytologia*, **6**: 422–430.

Vol. 85, No. 3 December, 1943

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE OSMOTIC PROPERTIES OF CYTOPLASMIC GRANULES OF THE SEA URCHIN EGG[1]

DANIEL L. HARRIS

(Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania, and The Marine Biological Laboratory, Woods Hole, Massachusetts)

INTRODUCTION

In many cells, a large part of the cytoplasm consists of numerous granules of various types. Undoubtedly they have a real functional significance, but little is yet known of the roles which they may play in cellular processes. This lack of knowledge may be due to the fact that, ordinarily, granules are rather inaccessible to experimental treatment. It was therefore thought worth while to attempt to isolate the granules and study them outside the living cell. This method has the advantage that it enables one to add reagents in known concentrations, and to be certain that they are affecting the granules directly and not secondarily through effects upon the cell itself.

Although most granules appear as solid particles, many may actually be minute vacuoles. That this is true of the pigment granules of the Arbacia egg was concluded by Chambers (1935) who found that the pigment escapes when these granules are punctured with a micro-needle. In preliminary studies, I was able to confirm this observation and to provide additional evidence that the pigment granules are actually vacuoles. The most cogent part of this evidence is the fact that in the presence of calcium, magnesium, and strontium ions, the pigment granules (and some colorless granules) coalesce with each other to form large fluid vacuoles from which the pigment soon diffuses, revealing small particles inside in active brownian movement. It is difficult to understand how two particles could coalesce in this manner unless they were vacuoles initially. Photomicrographs of this interesting reaction in isotonic $CaCl_2$ are shown in Figure 1.

Such minute granules or vacuoles should exhibit osmotic activity. That they do was concluded by Lewis and Lewis (1915) who actually observed swelling and shrinking of mitochondria in tissue culture cells placed in anisotonic media, and by Costello (1939) who found that the formed components of the Arbacia egg occupy 41 per cent of the total volume of the egg, while the osmotically

---

[1] Presented to the Faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

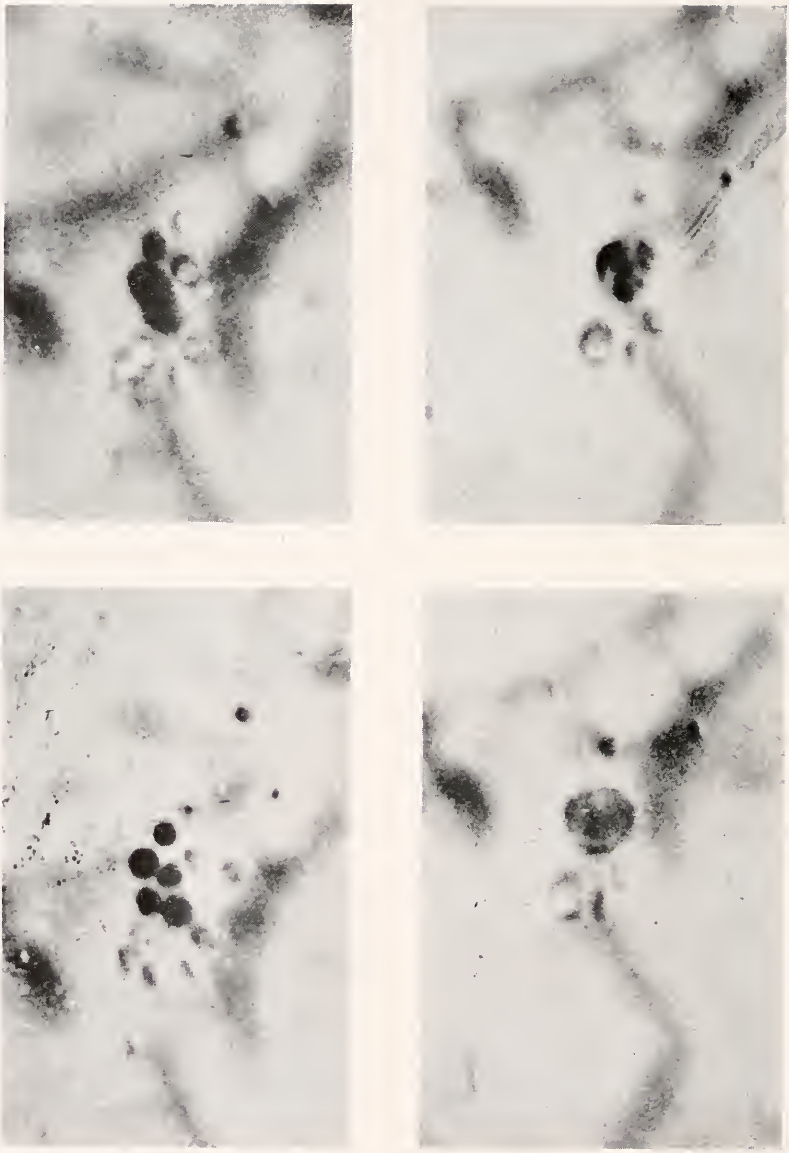


Figure 1. Coalescence of isolated vacuoles in calcium chloride. Upper left, five pigment vacuoles in center of photograph and three white granules above and to the left. Upper right, vacuoles coalescing. Lower left, coalescence complete, large pigment vacuole and large white vacuole. Lower right, pigment lost from vacuole, particles visible within large pigment vacuole.

inactive volume is only 7–14 per cent according to McCutcheon, Lucké and Hartline (1935).

The present paper presents direct evidence that the pigment vacuoles of the Arbacia egg are osmometers. They do not, therefore, constitute part of the osmotic dead space postulated by McCutcheon, Lucké and Hartline (1935). Agreement with the Boyle-Van't Hoff law is rather good but certain discrepancies point to the conclusion that osmotically active material, presumably salt, leaks out during the course of the swelling. Some data is given for other types of granular inclusions.

It is a pleasure to express my appreciation to Doctor L. V. Heilbrunn for his encouragement and stimulating advice.

Preparation of Material

Suspensions of granules or vacuoles *in vitro* may readily be obtained from sea urchin eggs if certain precautions are taken. The solutions used must be neutral or acid, isotonic, and free from calcium. In alkaline solutions or in hypotonic solutions the vacuoles undergo lysis. In solutions containing calcium (or magnesium or strontium in high concentration) the protoplasm escaping from a ruptured cell clots. This reaction, called by Heilbrunn (1928) the surface precipitation reaction, must be avoided, inasmuch as many vacuoles are trapped in the clotted protoplasm and others lyse or coalesce with each other. The necessary precautions may be conveniently taken by using an isotonic solution of sodium citrate (0.35 M). This solution has several additional advantages. It does not induce cytolysis and eggs washed in it become very fragile and easily ruptured.

Eggs were collected from 10–50 sea urchins by allowing them to shed into sea water. The shedding reaction was hastened by the addition of isotonic KCl to the exposed ovaries according to the method of Palmer (1937). The eggs were concentrated by centrifuging and washed with 0.35 M sodium citrate. After two washings, most of the eggs become very fragile and may be broken readily by squirting them in and out of a pipette. The vacuoles themselves are rather sensitive, and it was found impossible to rupture the most resistant eggs (about 25 per cent) without simultaneously destroying many of the vacuoles.

The crude suspension of all types of granules or vacuoles resulting from this treatment may be used for many experiments. However, if desired, the various components may be separated out by differential centrifuging. A wide range of conditions may be used to accomplish this; but, on the whole, it is somewhat better to use low centrifugal forces for a long time rather than high forces for a short time, since if the vacuoles become tightly packed in the bottom of the centrifuge tube it is difficult to re-suspend them without causing serious breakage. The following scheme has proved satisfactory. Unbroken cells are removed rapidly by filtering through coarse filter paper under light suction. The whole brei is then centrifuged with an International Centrifuge, size 1 type SB, or size 2. The pigment vacuoles are thrown down in about 30 minutes at 1000 rpm (189 × gravity, g). Yolk sediments at 2000 rpm (755 g) in one hour. At 3000 rpm (1698 g) very small particles are thrown down in considerable quantity in three to five hours. Fat granules rise to the top and are readily removed.

If centrifuging is prolonged or if higher forces are available, it is possible to obtain granule-free cytoplasm. In each fraction there is a certain amount of contamination. This may, for the most part, be removed by re-suspending the particles in fresh citrate and repeating the original centrifuging. After separation, the granules or vacuoles are washed with fresh isotonic citrate to remove traces of non-granular protoplasm.

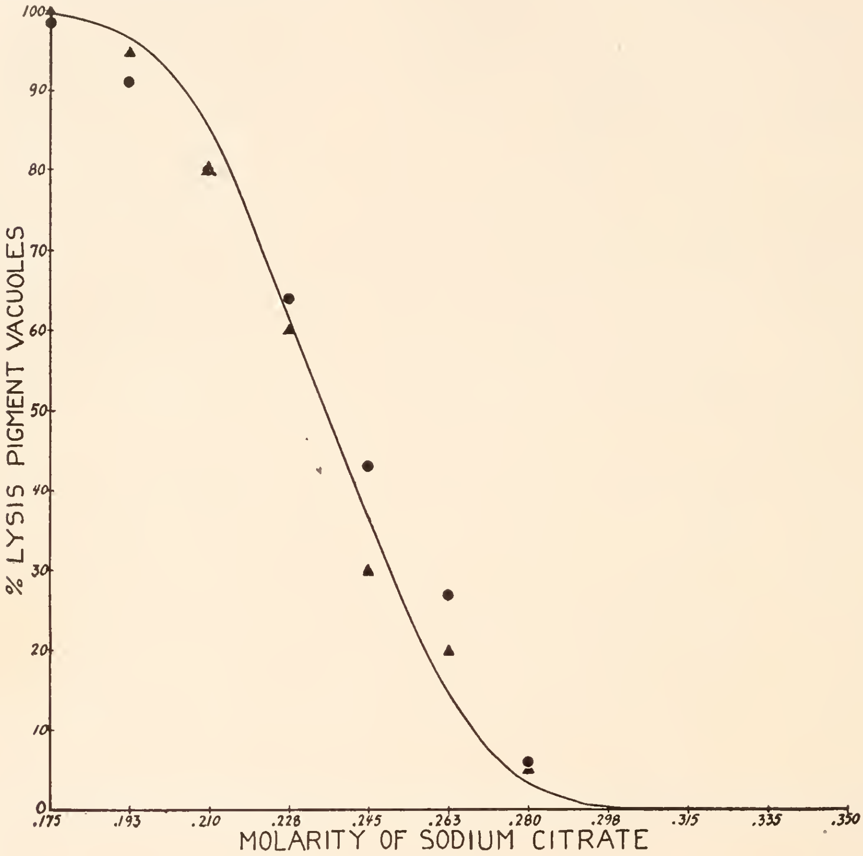


FIGURE 2. Lysis of pigment vacuoles in hypotonic sodium citrate. Circles represent data obtained by counting vacuoles microscopically; triangles, data obtained by colorimetric method explained in text. Curve is a cumulative projection of a normal or probability curve.

Another method of separating the constituents was sometimes employed. Concentrated solutions of sodium citrate may be prepared with a specific gravity higher than that of any of the granules, or with a specific gravity intermediate between that of any two types. By properly choosing the concentration, separation may be readily accomplished by placing a layer of concentrated citrate beneath the suspension of mixed particles before centrifuging. The proper concentration must be determined with each preparation because of variability in the specific gravity of the particles. Unfortunately, the pigment vacuoles undergo lysis with this treatment and, therefore, cannot be recovered intact. The yolk and the small granules do not seem to be adversely affected.

RESULTS

In hypotonic solutions pigment vacuoles undergo lysis. Microscopic observation of the reaction shows little detail, but it is possible to see that, after a short time, the pigment suddenly leaks out of the vacuole which then fades from view, leaving an indistinct ghost. There is no obvious rent in the vacuolar membrane, nor does the pigment stream out from a localized spot. On the contrary, it appears to diffuse through the entire membrane much as hemoglobin diffuses out of blood cells in hemolysis. Indeed, the phenomena of vacuolar lysis and hemolysis seem to be rather comparable, and the methods which have been used to study hemolysis may be applied here.

TABLE I

Times of lysis (seconds) of pigment vacuoles

Molarity Na_3 citrate		0.1750	0.1925	0.2100	0.2275	0.2450	0.2625
Osmotic pressure (Atmospheres)		10.50	11.49	12.47	13.46	14.44	15.43
Per cent lysis	Lytic osmotic pressure						
5	16.42	1.48	1.76	1.84	2.30	2.63	5.66
10	15.63	1.65	1.91	2.45	2.81	4.68	15.40
15	15.43	1.71	2.16	2.72	3.98	7.93	
20	15.04	1.80	2.88	3.72	4.85	14.50	
30	14.64	2.56	2.96	3.89	5.38	23.40	
35	14.44	3.54	5.15	6.51	10.30		
40	14.26	3.50	5.43	7.35	12.50		
50	13.95	6.83	7.80	12.70	28.70		
65	13.46	18.80	29.90	40.60			

If the vacuoles are osmometers, there should be more lysis in a very dilute solution than in a mildly dilute solution. This was studied in the following manner. Aliquots of the crude suspension were added to various concentrations of sodium citrate. Samples taken to determine the percentage of lysis were placed in a chamber of definite volume and uniform depth (Leitz dark field chamber) and the pigment vacuoles in a given area were counted. A 4 mm. objective and 10X ocular gave adequate definition. The results of these counts are represented by the circles in Figure 2. It will be noted that lysis increases as the external solution is made more dilute. Lysis is practically complete in 0.175 M sodium citrate. The curve is a cumulative projection of a probability curve, and the fit is close enough to indicate that the vacuoles are "normally" distributed in their resistance to lysis. The same type of curve is found in osmotic hemolysis, and is interpreted in the same way.

A quicker and easier method of estimating the amount of lysis is afforded by a colorimetric method. This depends upon certain properties of the pigment, echinochrome, contained within the vacuole. According to Kuhn and Wallenfels

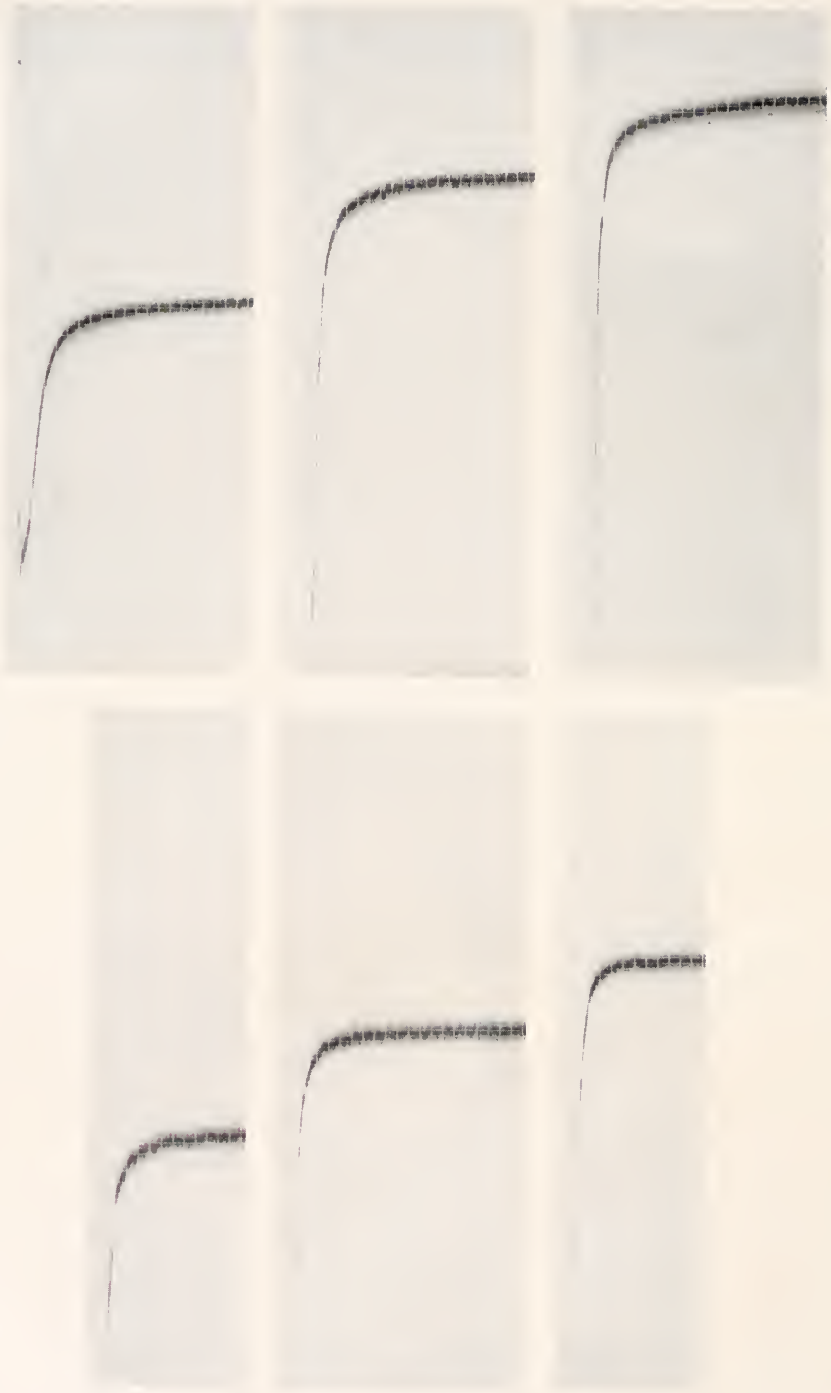


FIGURE 3.

(1939), this pigment is a polyhydroxyquinone bound to a protein. In the acid form it is red in color, and this is presumably the condition in which it exists within the vacuoles. The salts are variously colored. In sodium citrate at pH 7.4 the pigment escaping from the vacuoles turns first to a dirty brown and ultimately to a clear green. Intermediate colors are obtained which depend upon the amount of lysis. Standards may be prepared by making mixtures of suspensions of intact vacuoles with suspensions of vacuoles lysed with hypotonic citrate and brought back to isotonicity with concentrated citrate. The per-

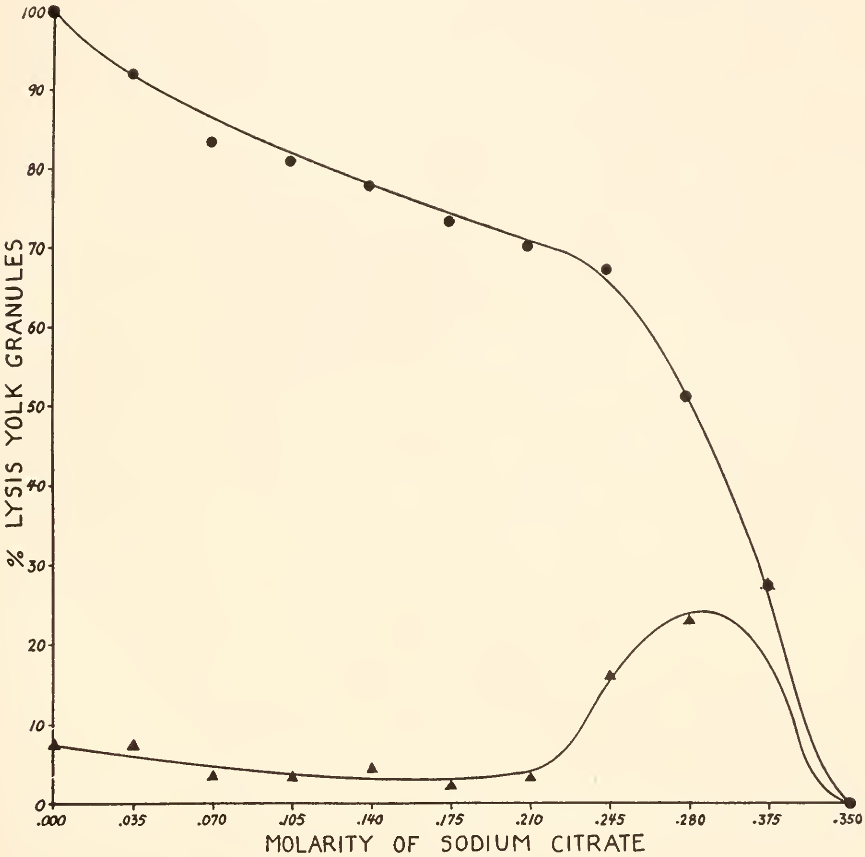


FIGURE 4. Lysis of yolk granules in hypotonic sodium citrate. Circles represent data obtained with the photoelectric method. Triangles show the increment of lysis with dilution. The curves are empirical.

FIGURE 3. Photographic records of granule lysis with Parpart's photoelectric method. The addition of the granules to the hypotonic solution is marked by a sudden drop in light transmission, here a thin vertical line (retouched). As lysis proceeds there is a rapid increase in light transmission followed by a slower increase to equilibrium. White vertical lines mark second intervals. Upper row: lysis of yolk granules in 0.280 M, 0.140 M, 0.035 M sodium citrate. Lower row: lysis of small granules (mitochondria?) in 0.280 M, 0.175 M, 0.105 M sodium citrate.

centage lysis in any experimental suspension may be determined by comparing the color with that of the standards. This was done at the same time the counts were made, and the results are shown by the triangles in Figure 2. Agreement between the two methods is good and the colorimetric method was used thereafter.

With the colorimetric method it is possible to study the kinetics of the reaction. The time taken to achieve a certain degree of lysis in a given hypotonic solution can be measured by determining the time required to reach a certain color. This was done with a stop-watch and visual inspection. The results are given in Table I. In this table the times required to attain different percentages of lysis in various concentrations of hypotonic sodium citrate are recorded. It is apparent that in the more dilute solutions, not only is the degree of lysis greater but the speed of the reaction is very much greater as well. In a more dilute solution the osmotic gradient is greater; water will therefore enter more rapidly; and the vacuoles will swell to the lytic size in a shorter time.

TABLE II

Times of lysis (seconds) of yolk granules

Molarity Na_3 citrate		0.00	0.035	0.070	0.105	0.140	0.175	0.210	0.245	0.280
Osmotic pressure (Atmospheres)		0.00	2.61	4.58	6.56	8.53	10.50	12.47	14.44	16.42
Per cent lysis	Lytic osmotic pressure									
27	18.38	0.34	0.41	0.46	0.41	0.62	0.76	1.02	1.46	6.50
51	16.42	0.44	0.56	0.68	0.75	1.07	1.42	2.07	5.90	
68	14.44	0.55	0.86	1.25	2.12	4.90				
71	12.47	0.58	1.05	1.91	4.80					
73	10.50	0.61	1.29	3.80						
78	8.53	0.66	2.68							
81	6.56	0.79	14.18							
86	4.58	0.87								
92	2.61	2.18								

A few observations were made of the lysis of purified yolk granules using the photoelectric method of Parpart (1935).[2] Light from a constant source is sent through a chamber and is measured by means of a Photronic cell and a Kipp-Mall galvanometer with photographic recording. Typical records are shown in the top row of Figure 3. On the addition of 50 mm.[3] of granule suspension to the dilute salt solution in the chamber there is a rapid drop in the light transmission. As lysis proceeds, more light passes through the suspension and the galvanometer tends to return to its original position. The results of these experiments are summarized in Figure 4 and Table II.

Similar records were made with the smallest granules (mitochondria?). Unfortunately, these particles tend to clump together and it is exceedingly difficult to obtain uniform samples. Analysis of the data is at present impossible. Typical records are, however, shown in the bottom row of Figure 3.

---

[2] I am very grateful to Doctor A. K. Parpart for the loan of his own apparatus.

Discussion

The pigment vacuole:

While the above data indicate clearly that the pigment vacuoles are osmometers, a closer analysis is desirable. We would like to know if the Boyle-Van't Hoff law is obeyed. Equations suitable for testing this have been derived by Jacobs (1932) for the comparable case of the osmotic hemolysis of blood. For the general case:

$$\frac{KAt}{V_0} = \frac{p_0}{p^2}\ln\frac{p_0p - pP}{p_0p - p_0P} + \frac{p_0}{P}\left(\frac{1}{p_0} - \frac{1}{p}\right)$$

and for the special case where cells (or vacuoles) swell in distilled water:

$$\frac{KAt}{V_0} = \frac{p_0}{2}\left(\frac{1}{p^2} - \frac{1}{p_0^2}\right).$$

In these equations, K is the permeability constant, a measure of the volume of water entering the vacuole through a unit area in a unit time under a unit osmotic gradient. A is the surface area, V_0 the initial volume of the vacuoles.

Table III

Permeability of the pigment vacuoles to water (K')

Molarity Na_3 citrate		0.1750	0.1925	0.2100	0.2275	0.2450	0.2625
Osmotic pressure (Atmospheres)		10.50	11.49	12.47	13.46	14.44	15.43
Per cent lysis	Lytic osmotic pressure						
5	16.42	.022	.021	.024	.024	.027	.019
10	15.63	.027	.027	.026	.029	.024	.016
15	15.43	.026	.026	.025	.022	.016	
20	15.04	.031	.023	.025	.023	.012	
30	14.64	.025	.026	.025	.025	.012	
35	14.44	.020	.016	.017	.010		
40	14.26	.021	.017	.016	.014		
50	13.95	.012	.013	.011	.008		
65	13.46	.005	.004	.004			

P is the osmotic pressure of the external solution, an experimentally controlled variable. The volume of the external solution is very large in comparison to the total volume of the vacuoles, so that the external osmotic pressure does not change during the course of an experiment. Now, p_0 is the osmotic pressure of the solution inside the vacuole initially, p the osmotic pressure inside the vacuole at time t. The osmotic pressure within the vacuole may be assumed to be that of the solution with which it is in equilibrium. Initially, this is equivalent to 0.35 M sodium citrate, or 20.36 atmospheres (calculated from data of Hitchcock and Dougan, 1935). We are interested only in a particular value of p, that is p_L corresponding to t_L. This is the osmotic pressure inside the vacuoles at the moment of lysis, and is presumed to be exactly equivalent to the osmotic pressure of the solution which causes that degree of lysis, i.e., the solution with which it is in equilibrium.

For the purpose of determining agreement with the Boyle-Van't Hoff law, it is not necessary to know the exact volume, V_0, or surface area, A, since these are constant and may be combined with K, the true permeability constant, to give a new constant K'. If the same value of K' is found for all concentrations, it may be concluded that the vacuoles obey the Boyle-Van't Hoff law.

The data necessary for this calculation may be obtained from Figure 2 and Table I and the results are given in Table III for several concentrations and a number of degrees of lysis.

It will be noted that when 5 to 30 per cent lysis occurs, all the values of K' lie around 0.025 except for the last figure in each row, which is close to equilibrium and may be disregarded. From 35 to 65 per cent lysis, K' falls to very low levels. It may be further noted that in many of the rows there is a gradual decrease of K'. This is not especially serious except in more advanced degrees of lysis.

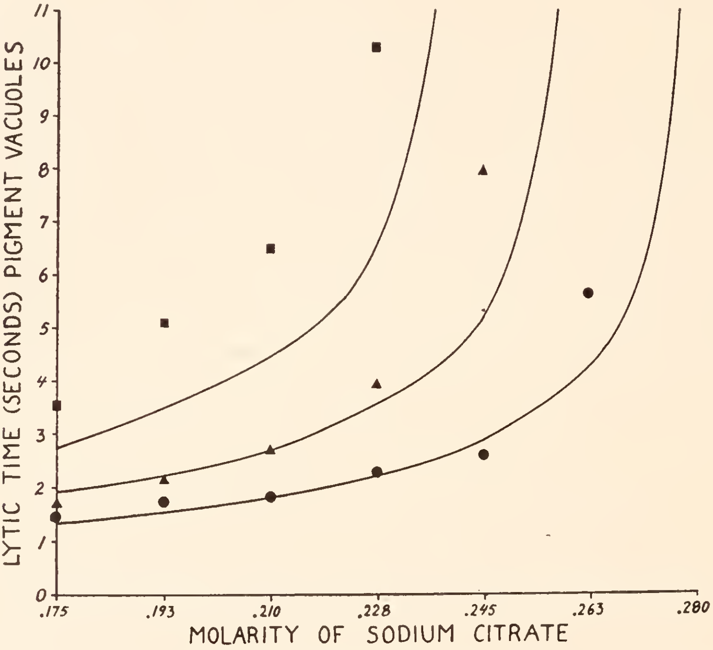


FIGURE 5. Lytic time of pigment vacuoles in hypotonic sodium citrate. Circles represent 5 per cent lysis, triangles 15 per cent lysis, and squares 35 per cent lysis. Curves are based on equation given in the text assuming $K' = 0.025$.

From these data it seems justifiable to conclude that up to 30 per cent lysis, K' is essentially constant and the pigment vacuoles therefore obey the Boyle-Van't Hoff law. This can be shown in a somewhat more convincing manner by a comparison of the observed rate of lysis with the theoretical rate, assuming a value of $K' = 0.025$. Figure 5 shows the theoretical curves for three different degrees of lysis. It will be noted that for 35 per cent lysis there is no agreement between the curve and the plotted data, although there would have been had another value of K' been chosen. Agreement of the data with the other two curves is good over a limited range, although large discrepancies exist at high concentrations of sodium citrate.

The reasons for the discrepancies which are found may now be considered. Either the permeability constant falls during the course of swelling or else the rate of lysis is very much slower for high degrees of lysis than is expected theoretically. With the data at hand, it is impossible to prove either of these possibilities, but the latter seems far more probable. The simplest interpretation is that osmotically active materials, presumably salts, leak out from the vacuoles, thus reducing the osmotic gradient and causing water to enter more slowly. This leakage of osmotically active materials does not include the echinochrome-protein complex. Obviously, within the cell the pigment does not normally diffuse out from the vacuole, or the vacuoles would contain no pigment. *In vitro*, the strict parallelism between the number of vacuoles lysed and the amount of pigment released as determined colorimetrically shows that there is no escape of echinochrome from the vacuoles until lysis occurs.

In deriving the equations, Jacobs made certain assumptions which have likewise been made here. It is instructive to consider these assumptions, particularly with the view of determining if any of them may aid in explaining the discrepancies. It is first assumed that the surface area of the vacuole is not changed during the course of swelling. It is clear that in the original permeability equation, $dV/dt = KA(p - P)$, the rate of swelling is directly proportional to surface area. Thus if there is any significant increase in surface area during swelling, the vacuoles ought to lyse more rapidly than expected after the initial stages. Actually, the rate is slower. This does not mean that increase in surface area does not occur; but if such increase does occur, it will not serve to explain the divergent results and it is therefore a refinement which can be neglected.

The assumption is also made that the initial volumes of the individual vacuoles are the same. This is by no means true since they vary in size from one to two micra in diameter. This variation should not affect the results unless there is a correlation between the initial size and the lytic concentration. Such a condition would be true if it were assumed that the vacuoles undergo lysis only when they swell to a lytic volume constant for all vacuoles. In that case the largest should undergo lysis first. Progressively smaller granules would be destroyed in the course of time in very dilute solutions. Remembering that $K' = KA/V_0$ it is apparent that if the diameter, which is a measure of both V_0 and A, is assumed larger than it actually is, K' will appear fallaciously low. This would be the situation for high degrees of lysis. A correction for varying initial volumes would, therefore, reduce some of the discrepancies. It would not, however, raise to the extent necessary the low values of K' found with high degrees of lysis, nor would it affect at all the drift of K' in high concentrations.

In the absence of concrete data, this possibility of explaining the discrepant results cannot be disproved. However, it seems unlikely that there is a strict correlation between initial volume and lytic concentration in view of the fact that the same type of curve as is shown in Figure 1 is obtained in hemolysis.

Erythrocytes are quite uniform in size. Furthermore, a simple calculation will show that no strict relation, such as Boyle's law, is likely to exist between initial volume and lytic concentration. If vacuoles two micra in diameter are assumed to break in 0.280 M sodium citrate, it may be calculated by Boyle's law that vacuoles one micron in diameter ought to lyse in 0.035 M. Actually, lysis is complete in 0.175 M.

It seems more reasonable to assume that the vacuoles have to swell a certain proportion of their original volume before lysis occurs than that the lytic volume is constant for all size vacuoles. On this assumption, the lytic concentration for all of the vacuoles would be the same. In view of these reasons for considering initial volume and lytic concentration independent, there seems no necessity for assuming that a correlation exists. In that event, a variation in the initial volume will have no effect upon K', and V_0 may legitimately be considered constant.

It is of some interest to calculate the true permeability constant. This may be very simply done if we remember that $KA/V_0 = K'$. Since the pigment vacuoles are spherical, both V_0 and A can be expressed in terms of the diameter, D, and multiplying by 60 to change seconds into minutes we obtain:

$$K = \frac{60K'\pi D^3}{6\pi D^2} = 10K'D.$$

Taking 0.025 as near the true value of K', and $D = 1 - 2\mu$, K falls in the range of 0.25–0.50 with an average value of 0.38 cubic micra of water entering the vacuole per square micron of surface area per minute per atmosphere difference in osmotic pressure.

These values of the permeability constant should not be taken too seriously. They are of interest only in indicating the order of magnitude. The initial osmotically active volume is not exactly known. Moreover, it should be noted that these measurements were made *in vitro* and in the presence of the citrate ion. Both of these conditions might well influence the permeability. Nevertheless, the permeability constant is probably somewhat higher than that of the cell as a whole. Lucké, Hartline and McCutcheon find values of 0.087 for endosmosis and 0.141 for exosmosis. The pigment vacuoles will tend toward osmotic equilibrium with the rest of the cell and cannot constitute part of the osmotic dead space postulated by McCutcheon, Lucké and Hartline to explain divergencies of the osmotic behavior of the Arbacia egg. This dead space probably consists of fat granules, dissolved proteins, or the membranes, etc., which surround the cell and the various formed components in it.

If the interpretation that the vacuoles are leaky is correct, they should be in equilibrium with the rest of the cell in regard to salts and organic substances, e.g., metabolites. The pigment vacuoles undergo lysis in solutions of urea, acetamide, sucrose, ethylene glycol, etc., indicating they are permeable to these substances. Whether these or other substances added to the cell would penetrate the cell and all its constituents at the same rate, cannot be answered until comparative figures of the permeability constants of these substances are available. This information may ultimately have important bearing on problems of cell metabolism. Further experiments are planned.

Yolk granules:

The interpretation of the behavior of the yolk granules in hypotonic solutions is difficult. An inspection of the curve given in Figure 3 indicates that the reaction proceeds in two stages. This is especially clear from the curve marked with the triangles which is the increase in lysis with each increment of dilution. It is possible that down to about 0.245 M, the yolk particles are lysed osmotically, but that in lower concentrations some other process is operating.

TABLE IV

Permeability of yolk granules to water (K')

Molarity Na$_3$ citrate		0.00	0.035	0.070	0.105	0.140	0.175	0.210	0.245	0.280
Osmotic pressure (Atmospheres)		0.00	2.61	4.58	6.56	8.53	10.50	12.47	14.44	16.42
Per cent lysis	Lytic osmotic pressure									
27	18.38	.0173	.0160	.0161	.0210	.0169	.0164	.0158	.0156	.0060
51	16.42	.0321	.0281	.0268	.0285	.0244	.0230	.0215	.0122	
68	14.44	.0475	.0346	.0273	.0196	.0106				
71	12.47	.0769	.0491	.0322	.0159					
73	10.50	.1234	.0691	.0289						
78	8.53	.1996	.0605							
81	6.56	.3211	.0220							
86	4.58	.6921								
92	2.61	1.1394								

The rate of the reaction increases with dilution, as shown in Table II. This is consistent with the osmotic hypothesis. However the results of calculating K' (Table IV) do not lend support to this hypothesis. K' does not have the same value for all concentrations and all degrees of lysis. Indeed, it ranges from 0.0060 to 1.1394 in a very regular manner. Seemingly a simple osmotic hypothesis cannot explain these results. Perhaps an actual solution of the yolk particles in hypotonic solution occurs.

SUMMARY AND CONCLUSIONS

A method has been developed with which it is possible to obtain relatively pure suspensions of cytoplasmic granules in good physiological condition. With these preparations, some of the properties of the granules have been studied. It seems clearly established that the pigment granules are actually vacuoles, and that they show osmotic activity. No certain conclusions can be drawn about the yolk granules.

During the first few seconds of swelling, the pigment vacuoles show rather good agreement with the Boyle-Van't Hoff law. However, as exposure to hypotonic solutions continues, the rate of lysis falls below the expected value. This is interpreted as due to a leakage of osmotically active materials, probably salts, from the vacuoles, thus reducing the osmotic gradient, and therefore causing water to enter more slowly. The permeability constant of the pigment vacuoles seems to be somewhat higher than that of the cell as a whole. The vacuoles therefore tend toward osmotic equilibrium with the rest of the cell at all times. They do not constitute part of the osmotic dead space found by McCutcheon, Lucké and Hartline in the Arbacia egg.

If the interpretation that the granules are leaky is correct, they will tend toward equilibrium with the rest of the cell in respect to salts and to organic substances, although they are not permeable to the echinochrome-protein complex.

This information may eventually be of considerable importance for our understanding of the intimate problems of cellular metabolism and activity. Ultimately, students of cellular permeability, metabolism, or many of the other problems of cell physiology will have to consider the individual properties of all the components of the cell.

LITERATURE CITED

Chambers, R., 1935. The living cell. Chap. I, Textbook of Biochemistry, Harrow and Sherwin, Philadelphia.

Costello, D. P., 1939. The volumes occupied by the formed cytoplasmic components in marine eggs. *Physiol. Zool.*, **12**: 13–20.

Heilbrunn, L. V., 1928. The colloid chemistry of protoplasm. Berlin.

Hitchcock, D. I., and R. B. Dougan, 1935. Freezing points of anti-coagulant salt solutions. *J. Gen. Physiol.*, **18**: 485–490.

Jacobs, M. H., 1932. Osmotic properties of the erythrocyte. III. The applicability of osmotic laws to the rate of hemolysis in hypotonic solutions of non-electrolytes. *Biol. Bull.*, **62**: 178–194.

Kuhn, R., and K. Wallenfels, 1939. Über die chemische Natur des Stoffes, den die Eier des Seeigels (Arbacia pustulosa) absondern, um die Spermatozoen anzulocken. *Ber. des. deutsch. chem. Ges.*, **72**: 1409.

Lewis, M. R., and W. H. Lewis, 1915. Mitochondria and other cytoplasmic structures in tissue culture. *Am. J. Anat.* **17**: 339.

Lucké B., H. K. Hartline, and M. McCutcheon, 1931. Further studies on the kinetics of osmosis in living cells. *J. Gen. Physiol.*, **14**: 405–419.

McCutcheon, M., B. Lucké, and H. K. Hartline, 1931. The osmotic properties of living cells (eggs of Arbacia punctulata). *J. Gen. Physiol.*, **14**: 393–404.

Palmer, L., 1937. The shedding reaction in Arbacia punctulata. *Physiol. Zool.*, **10**: 352–367.

Parpart, A. K., 1935. The permeability of the mammalian erythrocyte to deuterium oxide (heavy water). *J. Cell. Comp. Physiol.*, **7**: 153.

THE RADIOSENSITIVITY OF EGGS OF ARBACIA PUNCTULATA IN VARIOUS SALT SOLUTIONS[1]

KARL M. WILBUR[2,3] AND RICHARD O. RECKNAGEL

(*The Marine Biological Laboratory, Woods Hole; Department of Zoology and Entomology, The Ohio State University; and the Zoological Laboratory, University of Pennsylvania*)

A variety of experimental procedures has been shown to alter the sensitivity of cells to x-rays and radium. Resistance to radiation can be increased by a reduction of oxygen (Crabtree and Cramer, 1933; Mottram, 1935; Anderson and Turkowitz, 1941); by the use of appropriate concentrations of ammonia (Zirkle, 1936; Marshak, 1938); CO_2 and H_2S (Zirkle, 1936, 1940, 1941) and by addition of protein to the medium in which the cells are immersed (Evans et al., 1941). Conversely, certain agents increase the radiosensitivity of biological material (see Scott, 1937). The present study has been carried out to ascertain whether alteration of the salt environment, which will in turn cause changes in the ionic composition, and to some extent the colloidal state of the protoplasm, will influence the action of x-radiation on the living cell.

Three solutions have been used to alter the ionic composition of the egg: isotonic potassium citrate; a mixture of isotonic $MgCl_2$ and sea water; and a mixture of isotonic $CaCl_2$ and sea water. Potassium citrate is of particular interest in this connection in that it will remove a large part of the calcium from the cell and at the same time is relatively non-toxic. A further point of interest lies in its inhibition of the reactions initiated by ultra-violet light in the Nereis egg (Heilbrunn and Wilbur, 1937). Magnesium, like citrate, is inhibitory with respect to ultra-violet action (Wilbur, 1939). Calcium is antagonistic to both citrate and magnesium in many reactions of living material and so has been studied along with these two ions in the present work.

METHODS

Prior to irradiation 0.1 to 0.2 cc. of concentrated eggs was added to 40 cc. of the experimental solution or sea water for various periods. The eggs were then transferred to small plastic dishes for irradiation. Following irradiation 0.15 to 0.25 cc. of solution containing the irradiated eggs was placed in 250 cc. of sea water to remove the experimental solution; and approximately 6 minutes later the eggs were transferred to a second dish of sea water which contained sperm. The time required for 50 per cent of the eggs to complete first cleavage was determined by fixing samples at 2-minute intervals in 1 per cent or 2.5 per cent formaldehyde in sea water after examination of the eggs showed that cleavage had begun. In a few instances in which the cleavage time occurred very slowly

---

[1] A grant from the Graduate School of The Ohio State University is gratefully acknowledged.

[2] A portion of this work was carried out during the tenure of a Rockefeller Fellowship.

[3] Present address: Physiology Dept., Dalhousie University, Halifax, Canada.

samples were fixed at 3-minute intervals.[4] By this method one can estimate the time to 50 per cent cleavage in normal eggs within one or two minutes. After very large doses of x-rays many of the eggs show multipolar cleavage, and it is not always easy to decide the exact time at which the cleavage furrows have cut completely through the egg. In such cases determinations of the time of cleavage are accordingly somewhat less accurate. In most experiments the percentage of multipolarity was estimated for the control and experimentally-treated eggs. Only those batches of eggs were used which on fertilization showed well-lifted membranes on at least 95 per cent of the eggs. During treatment with experimental solutions and x-radiation the eggs were at room temperature, which varied from 21 degrees to 26 degrees. Fertilization and cleavage were carried out in a water bath at a temperature of 25.01 ± 0.06 degrees.

TABLE I

Effect of x-radiation on Arbacia eggs following treatment with 0.35 M potassium citrate

Exp. No.	Cleavage time of non-irradiated eggs			Cleavage time of eggs receiving 30,400 r			Cleavage time of eggs receiving 53,200 r		
	Eggs in sea water throughout	Eggs treated with potassium citrate		Eggs in sea water throughout	Eggs treated with potassium citrate		Eggs in sea water throughout	Eggs treated with potassium citrate	
		For 30 min.	For 60 min.		For 30 min.	For 60 min.		For 30 min.	For 60 min.
I	II	III	IV	V	VI	VII	VIII	IX	X
1.	45	43					207	166	
2.	41	42					118	110	
3.	41	43					148	130	
4.	42	42					175	150	
5.	44	43					176.5	152	
6.	44	42					159	147	
7.	45	44					208	186	
8.	43.5	59					174	159	
9.	44	43		172.5	171				
10.	42	41.5		136	138				
11.	45	43		128	124				
12.	44	46		145	140*				
13.	39	39		188	186				
14.	43.5		42	121		108	147		122
15.	41		42	120		122	152		139†
16.	50		49	141		124	164		161†
17.	44		47	128		124	155		138
18.	45		45	146		140	166		151

* Total cleavage 76%–79%.
† Total cleavage 85%–86%.

The following solutions were used: 0.35 M potassium citrate; $CaCl_2$-sea-water mixture consisting of two parts of sea water and one part 0.3 M $CaCl_2$; and a $MgCl_2$-sea-water mixture made up of equal parts of 0.3 M $MgCl_2$ and sea water. The calcium content of the $CaCl_2$-sea-water mixture is approximately 9.6 times that of sea water. The $MgCl_2$-sea-water mixture has a magnesium content 3.3 times that of sea-water. The pH of sea water was 7.9, and the pH of all experimental solutions was 7.6 ± 0.2.

---

[4] A very few times the small numbers of available eggs made it necessary to make counts on the living eggs.

The x-radiation was carried out with the dual tube self-rectifying outfit available at the Marine Biological Laboratory. The secondary voltage was 182 kv., and the current on each tube was 25 ma. The distance from the center of each target to the center of the material irradiated was 9.5 cm. The eggs were irradiated in small plastic dishes approximately 2 cm. in diameter. The depth of the solution containing the eggs was approximately 0.9 cm. Experiments 1 through 8 (Table I) were carried out at an output of 7,600 r per minute, while all other experiments were exposed at an intensity of 5,600 r per minute.

Viscosity was determined by means of an Emerson hand centrifuge at a centrifugal force of approximately 1960 × gravity (Wilbur, 1940).

RESULTS

Experiments with Potassium Citrate

Cleavage Time

Unfertilized eggs treated with 0.35 M potassium citrate for 30 and 60 minutes were given various doses of x-rays and returned to sea water within 30 seconds following irradiation. The well known effect of roentgen rays in delaying the cleavage time is shown in Table I. With a dose of 30,400 r the eggs which had been in potassium citrate for 60 minutes cleaved somewhat sooner than the sea-water controls in four of the five cases (columns V and VII). With 53,200 r in 12 of the 13 cases studied the citrated eggs cleaved several minutes sooner than those in sea water (columns VIII, IX and X); and the 30-minute citrate treatment was quite as effective here as the 60-minute treatment. Smaller doses of 3,800

TABLE II

Effect of x-radiation on Arbacia eggs treated with potassium citrate for 20 minutes prior to and 20 minutes following irradiation

Exp. No.	Cleavage time of non-irradiated eggs		Cleavage time of eggs receiving 15,200 r		Cleavage time of eggs receiving 30,400 r	
	Eggs in sea water	Eggs treated with potassium citrate for 40 minutes	Eggs in sea water	Eggs treated with potassium citrate	Eggs in sea water	Eggs treated with potassium citrate
1.	45 min.	45 min.	82 min. (for 63% cl.)	82 min. (for 63% cl.)	110 min.	103 min.
2.	46 min.	46 min.	91	87	128	125
3.	43	42	72	70	126	120
4.	45	47	82	81	125	117
5.	45	45	79	66	138	108
6.	45	45	115	102	170	154
7.	46	46	Exovates on nearly all. Poor cleavage	No exovates. 98% cleavage	Exovates on nearly all. Poor cleavage	Exovates rare. 100% cleavage
8.	43	43	79	78	128	116

and 15,200 r delay cleavage to the same degree in citrated treated eggs and eggs in sea water (not shown in table).

Eggs treated with potassium citrate for 20 minutes prior to the completion of irradiation and allowed to remain in citrate for 20 minutes following irradiation were also protected from the x-ray action to some degree. The effect is clear-cut with 30,400 r and is indicated in some cases at 15,200 r (Table II). Although a 30-minute treatment with citrate prior to and during x-radiation has little or no protective action for a dose of 30,400 r (Table I, columns V and VI) a 20-minute treatment prior to and during x-radiation and followed by an additional 20-minute immersion after irradiation may inhibit the x-ray action. The difference is not especially striking, and we should not care to stress the point on the basis of the evidence at hand. However, the data do suggest the interesting possibility that the x-ray effect can be inhibited somewhat by changing the ionic composition of the protoplasm *following* the period of irradiation.

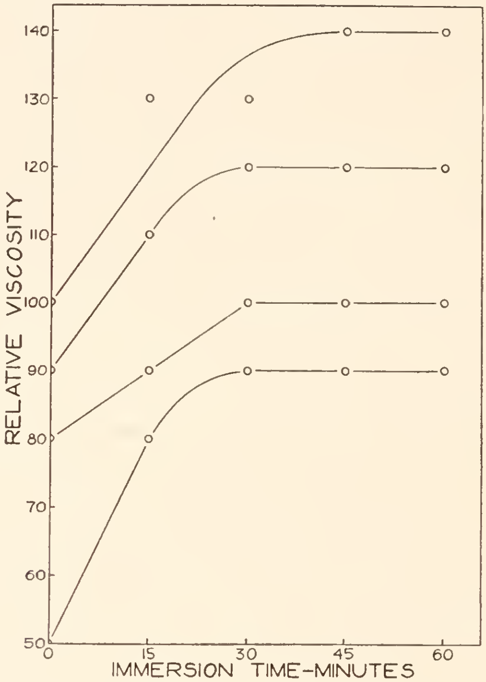


FIGURE 1. Viscosity of unfertilized Arbacia eggs in 0.35 M potassium citrate. The relative viscosity (ordinates) was measured following treatment in potassium citrate for various periods. (abscissas). pH 7.6 Temperature 24.0–25.2° C.

When eggs are x-rayed in sea water and immersed in potassium citrate immediately afterward, the citrate has no protective action. Five such experiments were carried out in which eggs were given doses of 15,200 and 30,400 r and changed from sea water to citrate in less than 30 seconds following irradiation, and immersed for 30 minutes. In this case some time would be required for equilibrium to be established between the citrate and the egg; and reactions initiated by the radiation may have gone to completion before the citrate exerted its full effect.[5]

Viscosity

The effect of 0.35 M potassium citrate on the colloidal state of the Arbacia egg at the time of irradiation as reflected in its viscosity has been studied. The viscosity changes of eight batches of eggs have been determined, and the results for four of these are shown in Figure 1. It is to be noted that potassium citrate causes an increase in viscosity. The highest value is usually reached in 30 minutes and maintained constant with continued immersion. Our concern has not been with the mechanism of the viscosity increase produced by potassium citrate. However, it may be pointed out that the potassium ion will in itself increase the viscosity of protoplasm (Heilbrunn, 1937). Mazia (1940) has found a marked decrease in the calcium content of Arbacia eggs treated with potassium citrate; and this has been confirmed by Miss Pauline Hamilton for the particular conditions of our experiments.

Experiments with Magnesium Chloride

Experiments similar to those with potassium citrate were carried out with a mixture of equal parts of 0.3 M $MgCl_2$ and sea water. The total period of immersion in the experimental solution was 60 minutes. The response to x-radiation of eggs treated with this mixture was much the same as in sea water. In each of six experiments doses of 15,200 r and 30,400 r were used. A dose of 53,200 r was employed in four experiments.

Viscosity determinations on eggs immersed for 55 minutes in the $MgCl_2$-sea-water mixtures revealed a slight decrease in seven of nine batches of eggs. The average decrease in viscosity for these seven samples was approximately 12 per cent.

Experiments with Calcium Chloride

The effects of x-radiation on eggs treated for 60 minutes with a mixture of one part 0.3 M $CaCl_2$ and two parts sea water were similar to those produced on eggs irradiated in sea water. Doses of 3,800 r, 15,200 r, 30,400 r and 53,200 r were used.

The $CaCl_2$-sea-water mixture resembles $MgCl_2$-sea-water mixture causing a slight decrease in the viscosity of unfertilized eggs. The average decrease for five batches of eggs was about 15 per cent after 60 minutes treatment.

The Viscosity of Unfertilized Eggs Following X-Radiation

In collaboration with Mr. Walter Wilson the viscosity of unfertilized Arbacia eggs has been studied after irradiation in sea water in order to ascertain whether roentgen rays will produce viscosity changes in the living cell. A dose of 30,400 r was employed and the viscosity determined 25 minutes following the completion of the irradiation or approximately 30½ minutes from the time that irradiation was begun. The viscosity determinations were carried out at 24.4–25.8° C. This dosage has a drastic effect upon cleavage. The average cleavage time for 21 experiments was 134 minutes as compared with 44 minutes for the non-irradiated control eggs. The majority of eggs receiving this dosage also exhibit multipolar cleavage. However, this relatively enormous dose failed to produce detectable changes in the viscosity of the egg (five experiments).

---

[5] Such an assumption, however, involves an apparent contradiction in that the possible enhanced effect resulting from leaving eggs in citrate for a 20-minute period following irradiation would argue that the x-ray effect was not complete shortly after irradiation. But the situation in which sea water replaces citrate is not necessarily comparable to the present one in which citrate replaces sea water.

The centrifuge method as used here would enable one to distinguish between a relative viscosity of 70 units and one of 60 units, for example. Our negative results therefore apply only to differences of this order of magnitude.

We are indebted to Dr. L. V. Heilbrunn for his co-operation and advice; to Miss Pauline Hamilton for her kindness in carrying out the calcium analyses; and to Dr. F. R. Hayes for helpful suggestions during the preparation of the manuscript.

Discussion

The data presented indicate that potassium citrate inhibits the effect of x-radiation on cell division. However, the inhibition is slight and appears only with high x-ray doses. The effect of the citrate treatment prior to irradiation is to increase the viscosity of the protoplasm and to reduce the calcium content. But it is also almost certainly true that immersion of a cell in potassium citrate upsets the entire ionic equilibrium of the cell and not merely the calcium content. In view of this, the influence of the potassium citrate treatment may involve substances other than calcium. Dale (1942) found that various substances, including sodium oxalate, sodium nitrate and sodium nitrite, would inhibit the destruction of d-amino-acid oxidase by x-rays. As yet, however, there is no justification for assuming enzyme inhibition by citrate in the case of the Arbacia egg.

The experiments with calcium-rich and magnesium-rich sea water together with the citrate experiments at lower x-ray doses indicate that the egg probably can tolerate a considerable change in ionic composition without an alteration in radiosensitivity. That the colloidal state of the protoplasm was affected by the addition of these ions is shown in most cases by a change in the viscosity which is increased by potassium citrate and decreased by sea water containing excess calcium or magnesium.

Experiments were described pointing to a possible action of potassium citrate after the period of irradiation. Even in those cases in which eggs were changed from citrate to sea water immediately following irradiation, some time would be required before equilibrium could be established. It may be true that the entire action of citrate is exerted after irradiation. If such is the case, one would have to assume that at least a portion of the x-ray action is indirect. That is, the x-radiation initiates a reaction which is partially inhibited in the citrated egg.

It is rather remarkable that the viscosity of the unfertilized egg is unchanged by doses of radiation which so greatly alter the rate and normal course of cell division. The direct coagulation of proteins as an explanation of the biological effects of roentgen rays would seem to be ruled out in the present study (see Zirkle, 1940).

We should like to suggest that the chief action of x-rays on the egg is the alteration of some system, perhaps enzymic, which comes into prominence after fertilization and is of particular importance for certain phases of mitosis. This explanation has also been suggested for colchicine which may be without effect on the viscosity of the unfertilized Arbacia egg, yet changes the viscosity of the fertilized egg and inhibits cell division (Wilbur, 1940). That radiation may interfere with cellular respiratory systems has been pointed out by several workers (see, for example, Crabtree and Cramer, 1933; Rudisill and Hoch, 1938).

We may call attention to the interesting fact that eggs can be treated for relatively long periods with isotonic potassium citrate or solutions of high calcium or magnesium content and yet on return to sea water they can be fertilized and will usually cleave at a normal rate. The citrate and magnesium treatments may, however, cause a slight amount of multipolarity.

SUMMARY

1. Treatment of Arbacia eggs with 0.35 M potassium citrate inhibited the retarding action of x-radiation on cell division. However, the inhibition by citrate was slight and appeared mainly with high x-ray doses (30,400 and 53,200 r).

2. The radiosensitivity of the egg was unaffected by increasing the calcium or magnesium content of the sea-water medium.

3. The potassium citrate treatment employed increased the viscosity of the unfertilized egg. The viscosity was decreased slightly in the sea-water solutions of increased calcium or magnesium content.

The data presented indicate that changes in the ionic composition and viscosity of the protoplasm may occur without altering the sensitivity of the egg to x-radiation.

4. Doses of x-radiation which markedly altered the rate and normal course of cell division produced no detectable change in the viscosity of the unfertilized egg.

LITERATURE CITED

Anderson, R. S., and H. Turkowitz, 1941. The experimental modification of the sensitivity of yeast to roentgen rays. *Amer. Jour. Roent.*, **46**: 537–541.

Crabtree, H. G., and W. Cramer, 1933. The action of radium on cancer cells. II. Some factors determining the susceptibility of cancer cells to radium. *Proc. Roy. Soc. London*, B, **113**: 238–250.

Dale, W. M., 1942. The effect of x-rays on the conjugated protein d-amino-acid oxidase. *Biochem. Jour.*, **36**: 80–85.

Evans, T. C., J. C. Slaughter, E. P. Little, and G. Failla, 1941. The influence of the medium on the radiosensitivity of sperm. *Biol. Bull.*, **81**: 291–292.

Heilbrunn, L. V., 1937. *An Outline of General Physiology.* W. B. Saunders Co., Philadelphia: 76–77.

Heilbrunn, L. V., and K. M. Wilbur, 1937. Stimulation and nuclear breakdown in the Nereis egg. *Biol. Bull.*, **73**: 557–564.

Marshak, A., 1938. Alteration of chromosome sensitivity to x-rays with NH_4OH. *Proc. Soc. Exp. Biol. Med.*, **38**: 705–713.

Mazia, D., 1940. The binding of ions by the cell surface. *Cold Spring Harbor Sympos.*, 8: 195–203.

Mottram, J. C., 1935. On the alteration in the sensitivity of cells towards radiation produced by cold and by anaerobiosis. *Brit. Jour. Radiol.*, **8**: 32–39.

Rudisill, H., and J. H. Hoch, 1938. How x-rays may kill cells. *Radiol.*, **31**: 104–106.

Scott, C. M., 1937. Some quantitative aspects of the biological action of x and γ rays. *Med. Res. Counc. Spec. Rep. Ser.*, No. 223.

Wilbur, K. M., 1939. The relation of the magnesium ion to ultra-violet stimulation in the Nereis egg. *Physiol. Zool.*, **12**: 102–109.

Wilbur, K. M., 1940. Effects of colchicine upon viscosity of the Arbacia egg. *Proc. Soc. Exp. Biol. Med.*, **45**: 696–700.

Zirkle, R. E., 1936. Modification of radiosensitivity by means of readily penetrating acids and bases. *Amer. Jour. Roent.*, **35**: 230–237.

Zirkle, R. E., 1940. The influence of intracellular acidity on the radiosensitivity of various organisms. *Jour. Cell. Comp. Physiol.*, **16**: 301–311.

Zirkle, R. E., 1941. Combined influence of x-ray intensity and intracellular acidity on radiosensitivity. *Jour. Cell. Comp. Physiol.*, **17**: 65–70.

THE DISTRIBUTION AND REPRODUCTION OF SAGITTA ELEGANS ON GEORGES BANK IN RELATION TO THE HYDROGRAPHICAL CONDITIONS

GEORGE L. CLARKE, E. LOWE PIERCE, AND DEAN F. BUMPUS

(Biological Laboratories, Harvard University and Woods Hole Oceanographic Institution) [1]

During the past few years an investigation has been undertaken of the factors underlying the productivity of Georges Bank, an extensive fishing area lying east of Cape Cod (Fig. 1). The economy of this area depends upon various interdependencies of the fish populations, the bottom fauna, and the plankton; and all are profoundly affected by the complex of strong currents and persistent eddies which are found on the Bank. In order to understand the essential ecological relationships, it is therefore necessary to gain a knowledge of both the hydrography and the biology of the waters of the region.

The present study of the abundance, distribution, and seasonal cycle of reproduction of the chaetognath, *Sagitta elegans*, was undertaken first because this species forms a prominent element in the zooplankton of Georges Bank, and second because Sagitta may be used as a "current indicator" to aid in unravelling the involved current system of the region. This species is a relatively large, easily recognized member of the zooplankton and its body length and maturity stage are readily determined. The life span of *Sagitta elegans* is sufficient to bridge periods of six weeks or more, with the result that in cases where observations are repeated each month, the same population may be identified from one cruise to the next. This condition presents a desirable contrast to more rapidly reproducing organisms, such as diatoms, in which populations of large dimensions may appear or disappear within a week or so.

Certain relatively recent investigations of the ecology of Sagitta in other regions are available for comparison, but none had the advantage of our quantitative collection method, nor the opportunity for revisiting as frequently over a two-year period an extensive net-work of stations as characterized the present undertaking. The breeding and growth of *Sagitta elegans* was studied by Russell (1932; 1933) off Plymouth, England, and by Pierce (1941) in parts of the Irish Sea. *Sagitta elegans* has been employed successfully as a current indicator in British waters by Russell (1939) and the distribution of the species by currents in the Gulf of Maine has been critically investigated by Redfield and Beale (1940).

COLLECTION AND ANALYSIS OF MATERIAL

Samples of plankton and hydrographic data for the present study were obtained from the research vessel, "Atlantis", during eleven cruises to Georges Bank from September 1939 to June 1941 (Table I). On each cruise a net-work of 21 to 52 stations was occupied over the Bank. In all cruises (except that of

---

[1] Contribution No. 328.

January, 1940) the stations were ordinarily placed at 15-mile intervals on five or six parallel sections, about 25 miles apart, running SE and NW across the Bank and into the immediately adjacent waters. The location of the stations is indicated in the charts showing the distribution of Sagitta (Figs. 5 and 6). The stations covered the region from South Channel on the southwest to the eastern tip of Georges Bank and from the deep basin of the Gulf of Maine on the northwest to the edge of the continental shelf on the southeast. No stations could be made in the immediate vicinity of Cultivator and Georges Shoals. The segment of the ocean covered by the station net-work of each cruise was more than 150 miles long and 100 miles wide, or an area larger than the states of Massachusetts, Connecticut, and Rhode Island combined (Fig. 1).

TABLE I

List of cruises to Georges Bank

Cruise no.	Date	No. of stations	Station serial nos.
89	Sept. 6–13, 1939	52	3629–3680
93	Jan. 4–11, 1940	21	3726–3746
95	Mar. 21–Apr. 2, 1940	35	3792–3826
96	Apr. 17–27, 1940	26	3827–3852
97	May 9–16, 1940	33	3856–3888
98	June 1–8, 1940	36	3892–3927
100	June 19–27, 1940	36	3932–3967
112	Mar. 21–Apr. 2, 1941	33	4177–4209
113	Apr. 15–23, 1941	34	4210–4243
114	May 7–14, 1941	34	4244–4277
116	May 28–June 4, 1941	33	4278–4311

Standard hydrographic observations for salinity and temperature were made at every station and Secchi disc measurements of transparency were carried out during daylight stations. Studies of certain chemical characteristics of the water and of the phytoplankton population were undertaken by collaborating investigators (Sears, 1941; and Riley, 1941 and 1942).

The zooplankton was collected at each station by means of two or more hauls with Plankton Samplers (Clarke and Bumpus, 1940) and one haul with a stramin net. Sagittae were taken in adequate numbers in both types of equipment and the two sets of hauls served as a check on one another.

The opening of the Plankton Sampler, which is 12.7 cm. in diameter, is provided with a shutter, and each instrument contains a meter which records the amount of water filtered by the net. In the present case, the instruments were equipped with No. 2 silk nets (22 strands/cm.) and "oblique" hauls[1] were made at a speed of about 2 knots for periods of 25 to 40 minutes. Ordinarily between 10 and 20 cubic meters of water were filtered during each tow, but the action of the tide or of clogging was such that values as low as 5 m.^{3} and higher than 30 m.^{3} were recorded. This variation makes clear the need for measuring the amount of water which actually passes through the net. The Samplers were arranged vertically so as to divide the total depth of water into two or three strata and, when feasible, were attached to the same cable. The uppermost instrument sampled the "Shallow" Stratum, extending from a depth of 25 m. to the surface. The lower limit of this stratum corresponded roughly with the position of the thermocline in those areas where it existed. At stations where the water was less than 75 m. deep, the "Second-depth" Stratum extended from the bottom to 25 m. In water deeper than 75 m., however, the remaining distance to the bottom (or to a maximum depth of 200 m.) was divided into two equal parts and these comprised the "Second-depth" and the "Deep" Strata

---

[1] In an "oblique" haul the net is towed horizontally but is raised in steps so that the whole depth of the stratum concerned is sampled. The Sampler could be towed safely down to within three meters of the actual bottom.

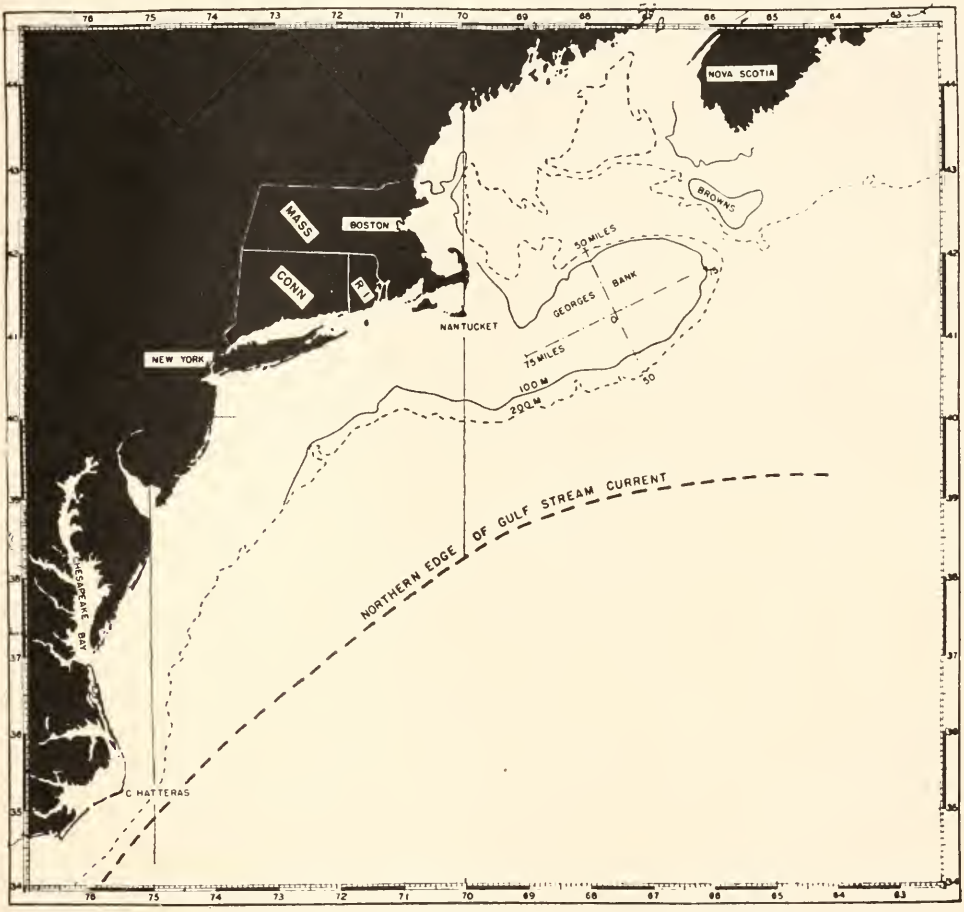

NOVA SCOTIA
BROWNS
BOSTON
MASS
CONN
R.I.
NEW YORK
NANTUCKET
50 MILES
GEORGES BANK
75 MILES
100 M
200 M
50
NORTHERN EDGE OF GULF STREAM CURRENT
CHESAPEAKE BAY
C HATTERAS
76
75
74
73
72
71
70
69
68
67
66
65
64
63

FIGURE 1. Orientation map of Atlantic Coast. The location and relative size of Georges Bank are indicated.

respectively. The vertical distribution of the sagittae could therefore be studied on the basis of these strata:

Stratum	Water less than 75 m.	Water more than 75 m.
"Shallow"	0 m. to 25 m.	0 m. to 25 m.
"Second-depth"	25 m. to bottom	25 m. to half distance to bottom (or to half distance to 200 m.)
"Deep"	——	Remaining distance to bottom (or to 200 m.)

The stramin net (Diameter: 1.5 m., Mesh: 6 strands/cm.) was equipped with rollers at the lower edge of its frame in order that it could be safely lowered until it touched the bottom. One "oblique" haul was made from the bottom (or from a depth of 200 m.) to the surface at each station. When proper allowance was made for the difference in the sizes of the apertures of the stramin net and the Plankton Samplers, a good agreement was found between the numbers of sagittae taken by the former and the sum of the catches of the latter at each station.

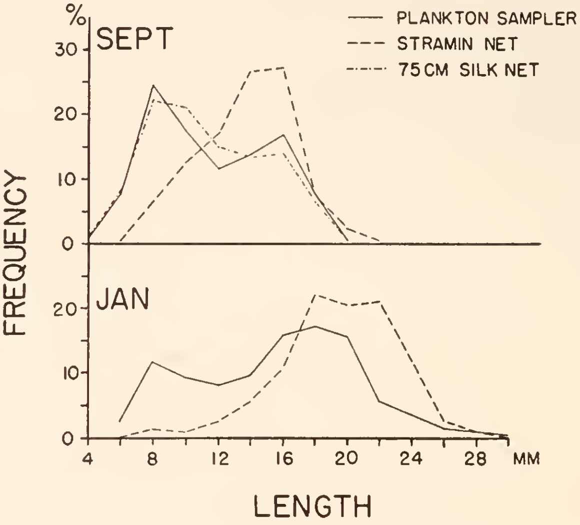


FIGURE 2. Comparison of length frequency distribution of *Sagitta elegans* for the following types of nets:

Plankton Sampler (12.7 cm. in diameter) with No. 2 silk.
Silk net (75 cm. in diameter) with No. 2 Silk.
Stramin net (1.5 m. in diameter).

Before the work was begun, it was doubted whether the relatively small Plankton Sampler would catch the larger sizes of an active animal, such as Sagitta, in their true proportions. For the first cruise (September 1939), therefore, a silk net 75 cm. in diameter and of the same mesh, was towed immediately below the Plankton Sampler. Since comparison of the length frequency distribution of the sagittae taken by the two sizes of nets showed exceptionally good agreement (Fig. 2), it is felt that the catch of the Plankton Sampler can be relied upon. In the January cruise sagittae as large as 30 mm. in length were retained by the Sampler. On the other hand, the stramin net was shown not to retain adequately the smallest sizes of sagittae. For these reasons and especially because of the accuracy of the determinations of depth and volume with the Plankton Samplers, the ensuing analysis of the abundance and distribution of the Sagitta populations is based primarily upon the hauls with these instruments. The present observations can therefore be placed on a quantitative basis not hitherto possible.

The sagittae were separated from the remaining plankton in the laboratory and the species present were identified and enumerated.[1] The great majority of sagittae were *Sagitta elegans* but specimens of *S. serratodentata* and a smaller number of *S. enflata* were encountered in certain hauls from the periphery of Georges Bank. For each station the average number of *S. elegans per cubic meter* was calculated *for each stratum* by dividing the number caught in each haul by the volume of water filtered by the Plankton Sampler. The total number of individuals *under each square meter* of sea surface was found by multiplying the number per cubic meter for each stratum by the thickness of the appropriate stratum and then adding these products together. Finally the *average number of animals per cubic meter for the whole water column* at each station was obtained by dividing the foregoing value by the total depth of water at each station. These average values per cubic meter (or per ten cubic meters) have been plotted on the charts showing quantitative distribution (Figs. 5, 6, and 10), but they may readily be re-converted to the "per square meter" basis by multiplying by the depth in each case.

Length measurements were made of all specimens of *Sagitta elegans* in each haul up to a maximum of 50. The stage of maturity was also determined for the individuals of this species in most of the hauls in each cruise from all parts of the Bank. Animals from each haul (usually between 20 and 50 individuals) were stained by the method described by Pierce (1941, p. 115), and then were classified as Stage I—*Immature*, Stage II—*Intermediate*, or Stage III—*Mature*, following the criteria of Russell (1932, p. 134).

General Hydrographical Conditions

The depth of the major portion of Georges Bank lies between 40 m. and 100 m., although areas of less than 25 m. occur in the north central portion, and the Shoals themselves are covered by only 5 to 15 m. of water. Along the northern edge of the Bank the bottom drops rapidly from about 40 m. to more than 200 m. as the deep basin of the Gulf of Maine is approached. Along the southern edge the depth changes somewhat more gradually from 100 m. to 200 m. Beyond 200 m. it increases rapidly to about 2000 m.

Georges Bank is therefore, roughly speaking, a submerged, flat-topped plateau (Fig. 3), and it presents a sufficiently large obstacle to water movement to produce a profound effect on the ocean currents of this region. Although the details of the water movements over and around the Bank have never been adequately determined, especially for the colder part of the year, it has been well established in general that during the summer months at least, water from the Gulf of Maine

[1] The authors are indebted to Miss Dorcas Delabarre for technical assistance in the analysis of the Sagitta material.

does not flow directly across the Bank but tends to move around the eastern and southern margins of the Bank in a clockwise direction, leaving a relatively stationary eddy of water over the central part of the Bank. From the point of view of the ecology of the Bank, our interest in the current system lies in the question of the degree of permanence of this eddy, and in the extent to which the "bank water" can be regarded as biologically isolated from the surrounding regions.

The eddy on the Bank might be dislodged by relatively slight changes in the strength or position of the surrounding ocean currents (Iselin, 1939), or it might be disrupted by the action of certain local agents. The strong tidal currents on the Bank cause the overlying water to oscillate in generally elliptical paths, the long diameters of which may exceed eight miles. Winds, which frequently reach gale velocities, sweep unimpeded across the area, tending to force the surface water along with them. The danger would thus appear to exist that from time to time the bank water might be swept entirely off the Bank, carrying with it the pelagic stages of animals which, as adults, could live only in a bank environment, or removing an element of the plankton which is essential to the economy of the Bank. Even though no cataclysmic dislocation of the bank water occurred, it is important to know to what extent a dilution or a renewal of the water mass may take place through continuous or intermittent admixtures of new water from one direction or another.

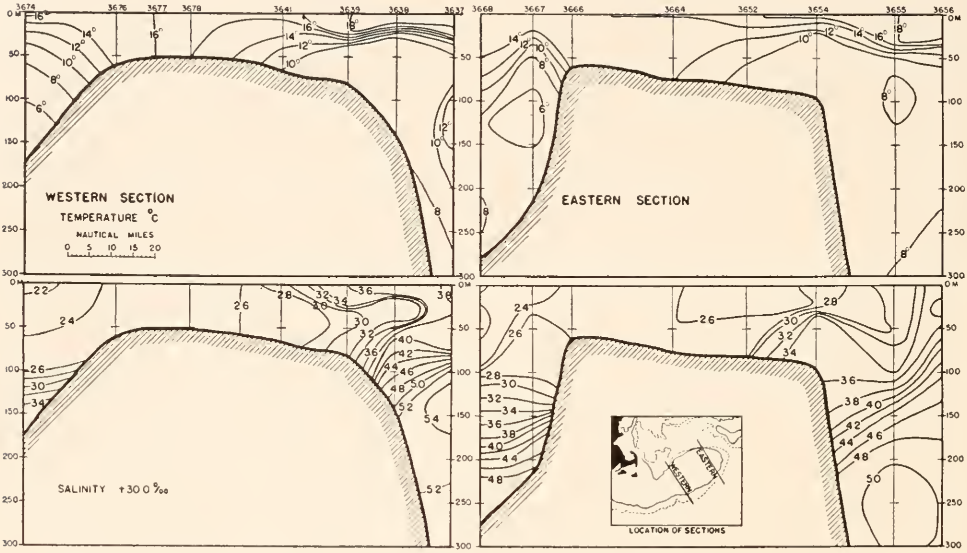


FIGURE 3. Vertical sections for temperature and salinity for September 1939 (cruise 89). The contour of Georges Bank is indicated by the cross hatching from the Gulf of Maine on the left to the edge of the Continental Shelf on the right. Station numbers appear at the top of the diagrams. The figures on the salinity curves are to be increased by 30 to give the actual values in parts per mille.

The turbulence produced by the tidal currents and by the wind in the relatively shallow water overlying Georges Bank causes a vertical mixing of the water which results in a nearly uniform distribution of temperature and salinity from top to bottom at all seasons of the year, particularly in the central part of the Bank. The bank water thus contrasts sharply with the surrounding water masses, which are typically stratified during all except the winter months. Since the temperatures and salinity values on the Bank are generally intermediate between those of the surface and the deeper strata on the Gulf of Maine but usually much lower than those of the water lying to the south, we know that the bank water is originally derived, in a large part at least, from the Gulf (Figs. 3 and 4). That portion of the Bank over which vertically uniform water was found is termed the *Mixed Area*, and all stations at which the salinity does not vary by more than 0.2 part per mille from surface to bottom are considered to lie within it.[1] The limits of the Mixed Area are ordinarily rather sharp, and have been indicated by a heavy broken line in the charts of Sagitta distribution for each cruise (Figs. 5, 6, and 10).

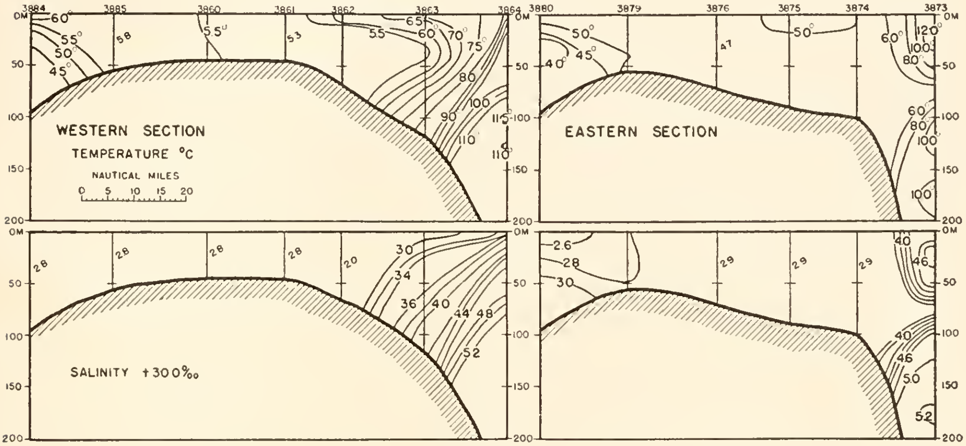


FIGURE 4. Vertical sections for temperature and salinity for May 1940 (cruise 97).

The vertical uniformity of the temperature and salinity within the Mixed Area presents an ecological condition for the Bank organisms which is quite unlike that for the oceanic forms living in the stratified water of the adjacent deeper areas. Moreover, seasonal changes in these factors are somewhat damped by the continued vertical mixing, as is seen by reference to Tables II and III. The seasonal range in temperature for the Mixed Area extended from a minimum of about 2.5° C. to a maximum of over 16° C. Surface temperatures in the regions to the north and to the south of Georges Bank were generally higher in summer and lower in winter. Furthermore, during the winter pelagic animals living at the surface in these neighboring areas could find warmer water by descending to lower strata, and similarly during the summer they could escape excessively high temperatures by seeking greater depths. In contrast, the fauna

---

[1] Uniformity of salinity, rather than temperature, was taken as the criterion for the Mixed Area because in the present situation salinity is less easily modified after the water has reached the Bank.

Table II

Comparison of temperatures in the Mixed Area and in the Stratified Water at the margins of Georges Bank

Values given are for typical stations near the center of the Bank and in the deeper water to the north and to the south. At the stations in the Mixed Area temperatures did not vary by more than 1.6° C. from surface to bottom and in most cases the variation was much less.

Date	Mixed Area	North Margin		South Margin	
	Aver. temp.	Surface	100 m.	Surface	100 m.
Sept., 1939	16.3° C.	16.4° C.	6.3° C.	19.3° C.	9.3° C.*
Jan., 1940	4.1	3.3	6.8	4.6	6.2*
Mar., 1940	2.5	2.1	5.3	2.8	3.9*
Apr., 1940	3.7	3.5	3.4	4.7	11.2
May, 1940	4.8	5.3	4.1*	6.1	7.8*
June 1–8, 1940	7.2	10.4	3.0*	10.8	9.3
June 19–27, 1940	8.5	10.6	3.1	10.3	10.2
Mar., 1941	2.7	3.3	4.1	2.9	4.9
Apr., 1941	3.9	4.3	4.5	3.2	4.9
May, 1941	4.6	6.5	5.4	4.0	4.5
June, 1941	6.8	9.1	3.6	7.3	5.4

* Value at somewhat less than 100 m.

Table III

Comparison of salinities in the Mixed Area and in the Stratified Water at the margins of Georges Bank

Values given are for typical stations near the center of the Bank and in the deeper water to the north and to the south. At the stations in the Mixed Area salinities did not vary by more than $0.2^{0}/_{00}$ from surface to bottom.

Date	Mixed Area	North Margin		South Margin	
	Aver. salinity	Surface	100 m.	Surface	100 m.
Sept., 1939	$32.5^{0}/_{00}$	$32.1^{0}/_{00}$	$32.5^{0}/_{00}$	$33.4^{0}/_{00}$	$33.8^{0}/_{00}$*
Jan., 1940	32.8	31.5	33.7	32.9	33.2*
Mar., 1940	32.8	32.4	33.2	32.7	33.0*
Apr., 1940	33.0	32.5	33.1	33.0	35.3
May, 1940	32.8	32.5	33.2*	32.7	34.1*
June 1–8, 1940	32.8	31.8	32.9*	33.1	33.5*
June 19–27, 1940	32.7	32.0	32.9	32.5	34.4
Mar., 1941	32.7	32.8	33.1	32.7	33.4
Apr., 1941	32.5	32.5	33.2	32.1	33.5
May, 1941	32.5	32.3	33.4	32.1	33.4
June, 1941	32.7	32.3	32.8	32.6	33.5

* Value at somewhat less than 100 m.

of the central bank waters could reach a materially different temperature only by migrating laterally entirely out of the Mixed Area. A similar situation obtains in regard to salinity, although there is little evidence that changes in salinity, *per se*, of the magnitude encountered in this region are of ecological importance. On the other hand, differences in density, which result in large part from the salinity, are bound to be critical for passively floating organisms, and the lack of a pronounced vertical density gradient in the Mixed Area, as well as the excessive turbulence there, presents a very special problem for such forms.

Quantitative Distribution of Sagitta Elegans

Vertical Distribution

The numerical abundance of *Sagitta elegans* varied greatly among individual hauls, ranging from a maximum of 165 specimens per cubic meter[1] to zero. When the hauls of each cruise are considered together, however, certain general trends in the changes in the Sagitta population become clear. The changes in horizontal distribution from cruise to cruise will be presented in the next section. In this section the variations in the vertical distribution will be considered as derived at each station from the separate hauls with the closing Plankton Samplers for the Shallow Stratum, the Second-depth Stratum, and the Deep Stratum. At stations where the depth of water was less than 75 m. the Second-depth haul extended to the bottom. Since this situation obtained at the majority of stations within the Mixed Area, the chief comparison for vertical distribution is between the Shallow Stratum and the Second-depth Stratum.

It is obvious that vertical distribution at stations made during the day (between the hours of sunrise and sunset) had to be distinguished from the situation at stations made during the night, since a diurnal migration of the animals was to be expected (Russell, 1933). Furthermore, if a vertical migration of the Sagitta tended to take place, very different conditions would be met with according to whether the station was in the Mixed Area or in the Stratified Area. If the animals encountered a thermocline, their movement might be stopped, or reversed (cf. Clarke, 1934). For the foregoing reasons the hauls upon which the analysis of the vertical distribution of *Sagitta elegans* is based, have been subdivided into those made at stations in the Mixed Area and those made at stations in the Stratified Area and have been further subdivided in each case into day and night hauls.

The *average* abundance of *Sagitta elegans* for all stations in each of these categories varied considerably from cruise to cruise (Table IV). In September the larger number of animals was found in the Second-depth Stratum in all cases, although at night in the Mixed Area an almost equally great number was taken at the upper level. In the winter and early spring of 1940 much smaller average numbers of sagittae were encountered and the differences in the various strata were not large. A tendency for the largest hauls to occur in the Deep Stratum is to be noted for May 1940, but this generality does not hold for the more sizable catches of the June cruises of that year. During the early spring of 1941 small numbers of sagittae were again encountered and their vertical distri-

---

[1] Shallow stratum haul, May 30, 1941, made in south central part of Bank and consisting of very small individuals.

TABLE IV

Numerical abundance of S. elegans *in the separate strata. Average number per cubic meter in the indicated categories.*

X indicates an abundance of less than 0.1/m^3. Values placed in parentheses are based on a total of less than 5 hauls.

Month	Cruise	Stratum	Mixed Area		Stratified Area	
			Day	Night	Day	Night
Sept., 1939	89	Shallow	5.3	17.5	0.7	1.6
		2nd Depth	10.8	17.9	8.1	(8.5)
Jan., 1940	93	Shallow	2.4	6.8	——	0
		2nd Depth	3.8	7.6	——	X
		Deep	(0.5)	(0)	——	0
Mar., 1940	95	Shallow	2.4	1.8	0	X
		2nd Depth	2.4	1.1	0	0.1
		Deep	(1.9)	(0.2)	X	X
Apr., 1940	96	Shallow	1.8	2.2	X	(0)
		2nd Depth	3.6	1.4	0.2	(0)
		Deep	(2.8)	——	(0.3)	(0)
May, 1940	97	Shallow	2.5	5.1	0.2	0.6
		2nd Depth	6.2	5.1	0.2	0.3
		Deep	(9.0)	(1.0)	16.6	13.0
June 1–8, 1940	98	Shallow	16.9	3.3	4.5	(3.3)
		2nd Depth	40.4	50.1	3.8	(5.9)
		Deep	(13.1)	(25.0)	3.6	(8.7)
June 19–27, 1940	100	Shallow	35.6	20.8	5.3	17.4
		2nd Depth	58.6	44.5	1.8	9.5
		Deep	——	(58.4)	3.4	12.6
Mar., 1941	112	Shallow	2.4	2.2	0	0.8
		2nd Depth	2.5	2.8	0	0.8
		Deep	(1.1)	(1.2)	X	1.2
Apr., 1941	113	Shallow	2.1	0.8	0	0.4
		2nd Depth	3.2	1.9	0	0.9
		Deep	(1.3)	(0.1)	0.1	0.1
May, 1941	114	Shallow	1.4	0.6	0	0.3
		2nd Depth	3.5	0.6	X	X
		Deep	(0.6)	(0)	0.3	1.0
June, 1941	116	Shallow	46.9	14.6	2.5	0.7
		2nd Depth	45.6	17.8	1.4	1.9
		Deep	(3.4)	(45.8)	1.6	2.5
Averages		Shallow	10.9	6.9	1.3	2.3
		2nd Depth	16.4	13.7	1.6	2.5
		Deep	3.7	14.6	2.9	3.9

bution was found to be generally uniform. This situation also held for May of that year, but in June much larger catches were made especially in the two upper strata for the day hauls and for the Deep Stratum for the night hauls in the Mixed Area.

In order to ascertain what tendency existed toward vertical diurnal migration it is not satisfactory to employ the foregoing average values because of the likelihood that a few large hauls would obscure differences occurring in stations with smaller representation. Accordingly a calculation has been made of the percentage of stations in each category for each cruise at which the number of *Sagitta elegans* in the Second-depth haul was greater than in the Shallow haul (Table V). When the data are scrutinized on this basis, it becomes clear that a definite vertical migration was taking place in both the Mixed Area and the Stratified Area at the time of the September cruise, since the majority of the animals were found below 25 m. in the day time and above 25 m. at night. A similar tendency, but less marked, was encountered in the first four cruises of 1940 and in the May cruise of 1941 for the Mixed Area. A reversal of the situation is to be noted for the June cruises in both 1940 and 1941, for in those cases the Second-depth hauls were greater at night at more stations than during the day. Taking the average for all cruises it is apparent that in all situations the deeper hauls were numerically greater in more than 50% of the cases. However, the variations encountered in vertical distribution and in diurnal migration from cruise to cruise show that the reactions of Sagitta in maintaining its vertical position in the water change materially according to the season or in relation to size and stage of maturity. A similar conclusion was reached by Russell (1933).

TABLE V

Comparative vertical distribution of S. elegans *for day and night hauls*

Percentage of Stations at which the number of animals per cubic meter in the "Second-Depth" haul was greater than in the "Shallow" haul. Values placed in parentheses are based on a total of less than 5 cases.

Month	Cruise	Mixed Area		Stratified Area	
		Day	Night	Day	Night
Sept., 1939	89	100%	29%	93%	50%
Jan., 1940	93	60	57		(100)
Mar., 1940	95	64	43		(67)
Apr., 1940	96	71	25	(100)	
May, 1940	97	86	50	60	(0)
June 1–8, 1940	98	70	71	(67)	(67)
June 19–27, 1940	100	75	100	20	33
Mar., 1941	112	78	70		(50)
Apr., 1941	113	73	(75)		(100)
May, 1941	114	75	(50)	(100)	(100)
June, 1941	116	46	80	(25)	50
Averages		73%	59%	66%	62%

Horizontal Distribution Throughout the Year

The quantitative aspects of the distribution of *Sagitta elegans* will be examined in relation to the location of the water masses on Georges Bank for September 1939 (Fig. 10), for the winter and spring of 1940 (Fig. 5), and for the spring of 1941 (Fig. 6). In each chart the station positions are designated by black dots and the *average* number of sagittae per 10 cubic meters *for the whole water column*

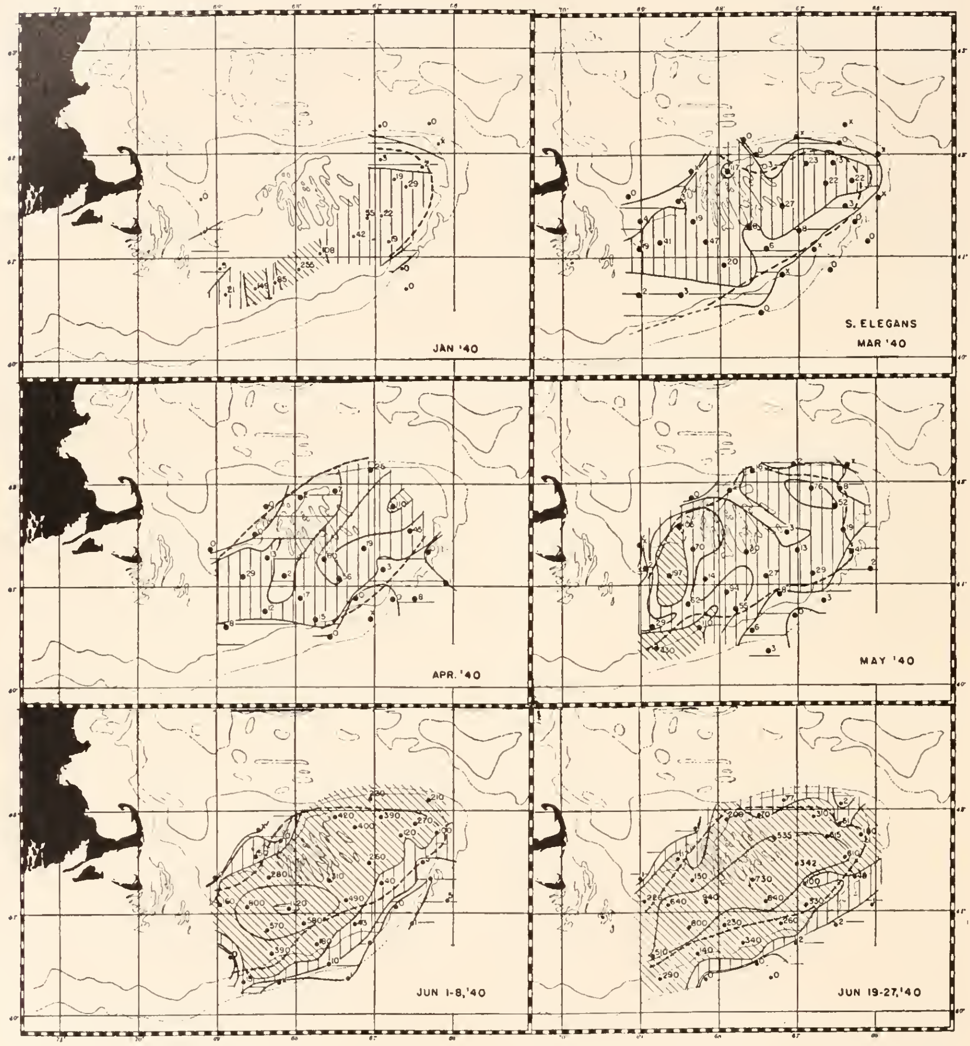


FIGURE 5. Distribution of *Sagitta elegans* on Georges Bank during 1940. January (cruise 93), March (cruise 95), April (cruise 96), May (cruise 97), June 1–8 (cruise 98), and June 19–27 (cruise 100). Average numbers per 10 cubic meters for whole water column for all stages. Plankton Sampler hauls. Boundary of Mixed Area indicated by heavy broken line.

at each station is indicated.[1] Contour lines representing concentrations of 1, 10, (50), 100, (500) and 1000 individuals per cubic meter have been drawn in. Progressively dense cross-hatching indicates areas of increasing numerical abundance. In addition, the position of the margin of the vertically homogeneous water of the Mixed Area for each cruise has been indicated by a heavy broken line superimposed independently on each chart.

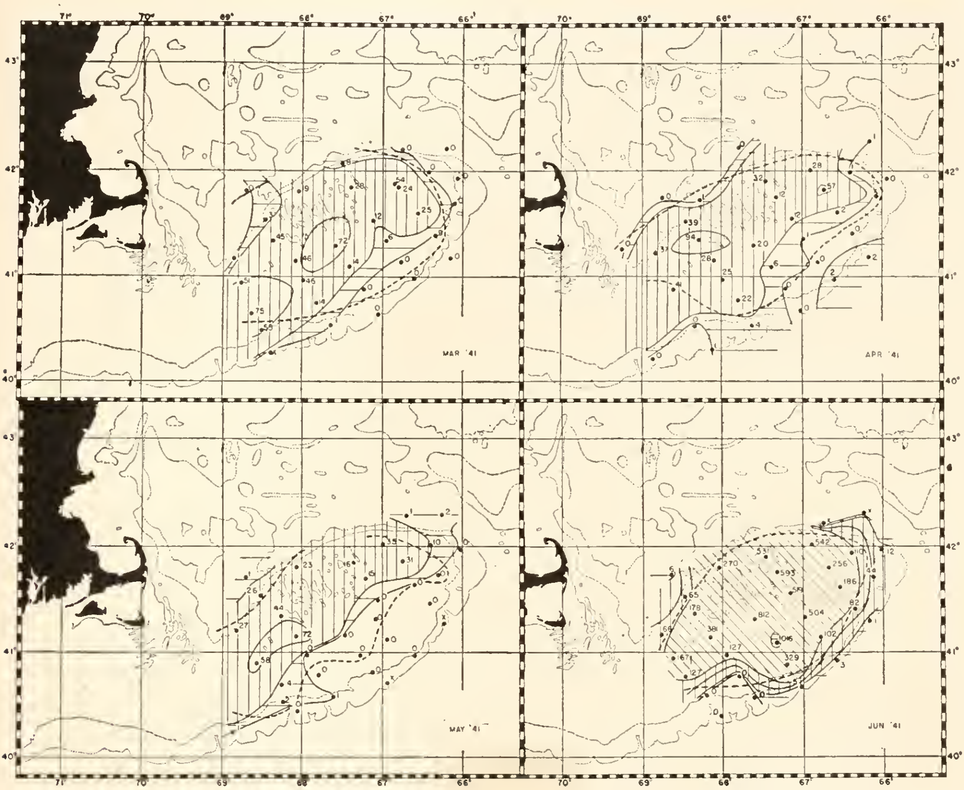


FIGURE 6. Distribution of *Sagitta elegans* on Georges Bank during 1941. March (cruise 112), April (cruise 113), May (cruise 114), and June (cruise 116). Average numbers per 10 cubic meters for whole water column for all stages. Plankton Sampler hauls. Boundary of Mixed Area indicated by heavy broken line.

Inspection of the chart for September 1939 (Figure 10, upper left) reveals the fact that at the time of this cruise extremely small numbers of *Sagitta elegans* occurred around the margins of the Bank (except possibly in the north central region). It is clear that the area of greatest concentration of this species lay in the center of the Bank. During this period the Mixed Area did not cover the whole of Georges Bank but was confined chiefly to the central portion except in the north where it extended beyond depths of 100 m. A rough correspondence is seen to exist for this cruise between the contour for 100 sagittae per 10 cubic meters (or 10 animals per cubic meter) and the limits of the Mixed Area.

---

[1] The total number of sagittae *under each square meter* of sea surface may be derived from these values by multiplying by the depth (see "Collection and Analysis of Material").

A similar scrutiny of the distribution of *Sagitta elegans* may be undertaken for the succeeding cruises from Figures 5 and 6. A great variation in the numerical strength of this species during the year is indicated by the fact that although numbers greater than 10/m.^{3} were encountered at nine stations in the September cruise, only one to four stations were as rich during the next four cruises. However, in each of the June cruises of 1940 about 20 stations yielded an average abundance of more than 10/m.^{3} and one station of more than 100/m.^{3} Similarly in 1941 during the first three cruises there were no hauls containing more than 10/m.^{3} but in the June cruise this value was exceeded at 18 stations and there was one instance of an abundance greater than 100/m.^{3} Although it was unfortunately not possible to make observations in every month of the year, as would have been desirable, the available information strongly indicates that the numerical strength of *Sagitta elegans* in the Georges Bank region reaches a low ebb in the winter and early spring, and attains high values beginning in June and perhaps extending through the summer.

In each of the eleven cruises the center of abundance of *Sagitta elegans* was found to be located within the central portion of the Bank and numbers dropped off toward the margin. Along the southern edge of the Bank the concentration of this species dwindled to a very small proportion and frequently to zero, especially beyond the 200 m. contour. Similarly low numbers were usually encountered along the eastern and northern margins although in some cruises an insufficient number of stations was occupied beyond the Bank to make certain of the limits of the population to the north. Since in most cases *Sagitta elegans* occurred in abundance at the westernmost stations of each cruise, we have definite indication that, at certain seasons of the year at least, numbers of this species are transported by westerly currents across South Channel toward Nantucket Shoals.

When the center of abundance of *Sagitta elegans* is compared with the location of the Mixed Area, it is clear that in spite of the changes in position of the latter from cruise to cruise, the greatest concentrations of the species were always found within the Mixed Area, and a close agreement existed between the contours of abundance and the boundary line of the mixed water (Figs. 5, 6, and 10). In addition to the situation in September, 1939, already described, striking cases of conformity between the distribution of the *S. elegans* population and the extent of the Mixed Area are seen in May and June (Fig. 6). During the May cruise stratified water was found to occupy the southern portion of the top of the Bank extending for 30 miles or more from the southern edge toward the center, whereas in June the mixed water largely covered this region. Corresponding to this shift in the position of the water masses *S. elegans* was found to be almost completely absent from the southern half of the Bank in May, but in June its distribution extended to the southern edge of the Bank.

In general the abundance of *S. elegans* tended to be relatively uniform for all the stations within the Mixed Area during each cruise. This fact showed that the spacing of the stations in this area was sufficiently close. The uniformity was no doubt due in large measure to the turbulence of the water in the Mixed Area and would not necessarily be expected in other regions of uniform hydrographic conditions but with less water movement.

Sagitta elegans is therefore chiefly abundant within the Mixed Area of Georges Bank, and during the periods covered by the present cruises, at least, this species appears to be largely isolated from surrounding regions. Evidence has been presented above that a small part of the population may be carried to the west at certain seasons by the movement of water around Nantucket Shoals. It is unfortunate that our observations could not have been extended to Cape Cod and to the waters north of the Cape in order to ascertain whether the sagittae of Georges Bank ever attain any important relationship with populations occurring in that region. As far as our present data go, however, no significant connection is indicated between the concentration of *S. elegans* in the vicinity of Massachusetts Bay reported by Redfield and Beale (1940) and the population on the Bank.

Growth and Breeding of Sagitta Elegans

Seasonal Changes in Length

The specimens of *Sagitta elegans* taken during the present investigation varied greatly in size, covering a range in length from 4 mm. to 30 mm. The frequency distribution of the sizes for all the hauls of the 1939–1940 cruises may be examined from the histograms of Figure 7. It is seen that no specimens longer than 20 mm. were taken in September, but individuals as long as 30 mm. occurred in January, March, and May. The modal length increased from 16 mm. in September to 18–20 mm. in January, and to 24 mm. in March. In April the modal length was 22 mm. These larger sizes were also represented in May and June, but in diminishing numbers.

Specimens of *Sagitta elegans* as small as 6 mm. were present in the September cruise in numbers nearly as great as the intermediate sizes. In January the presence of a secondary mode at 8 mm. suggests the simultaneous existence of two generations. In March and April, however, the smaller sizes were reduced to extremely small numbers and the intermediate sizes were poorly represented. Smaller individuals appeared in May and were more abundant in that month than the larger categories. By early June the numerical strength of this new crop of small Sagitta had increased ten fold and by late June they were still abundant. The modal length increased from 6 mm. in May to 8 mm. in June. Although in the late June cruise there were many more Sagitta in the 4 mm. class, an even greater augmentation of the sizes larger than 10 mm. was observed. Similar changes in the relative abundance and length distribution of this species were encountered during the cruises of 1941.

Scrutiny of the length distributions at the various individual stations and within the different strata revealed in general no tendencies for segregation. In most cases both large and small specimens were represented in the same relative proportions at the various depths at each station. Although considerable variation in length frequency occurred from station to station, nevertheless in most cruises there was no consistent tendency for large or small individuals to appear in certain parts of the Bank. An exception to the foregoing statement occurred in the cruise of May 1940, as shown in Figure 8, in which individual frequency distributions have been plotted for each station with sufficient numbers. In this case it is seen that at the easternmost stations only large specimens of

Sagitta elegans were taken, whereas at the western stations the smaller sizes definitely predominated. Evidently the remnant of the older animals persisted chiefly in central and eastern eddies, while the production of younger individuals was beginning most actively in the western parts of the Bank (see below).

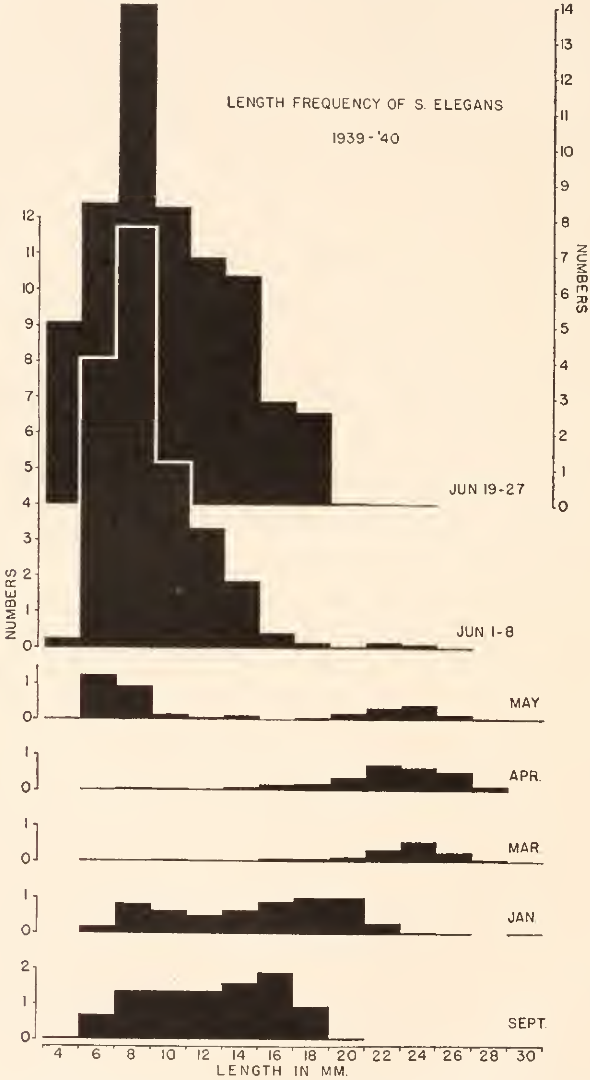


FIGURE 7. Average frequency distribution of lengths during 1939–40. Average numbers per cubic meter of *S. elegans* for each cruise.

Maturity Stages and Breeding Periods

Length measurements alone are not sufficient for determining the breeding periods of Sagitta because very great variations exist in the sizes of the three stages of maturity. This fact is amply demonstrated by the graphs of Figure 9, in which the length frequency distribution of each maturity stage has been plotted on a percentage basis for each cruise. Here it is seen that immature specimens (Stage I) may attain a length of 16 mm. or more, but individuals as short as 8 mm. may definitely have attained the "intermediate" condition (Stage II). Furthermore some specimens grew to lengths of 24 mm. or more while still in Stage II, while other individuals became completely mature (Stage III) at a length of 12 mm.

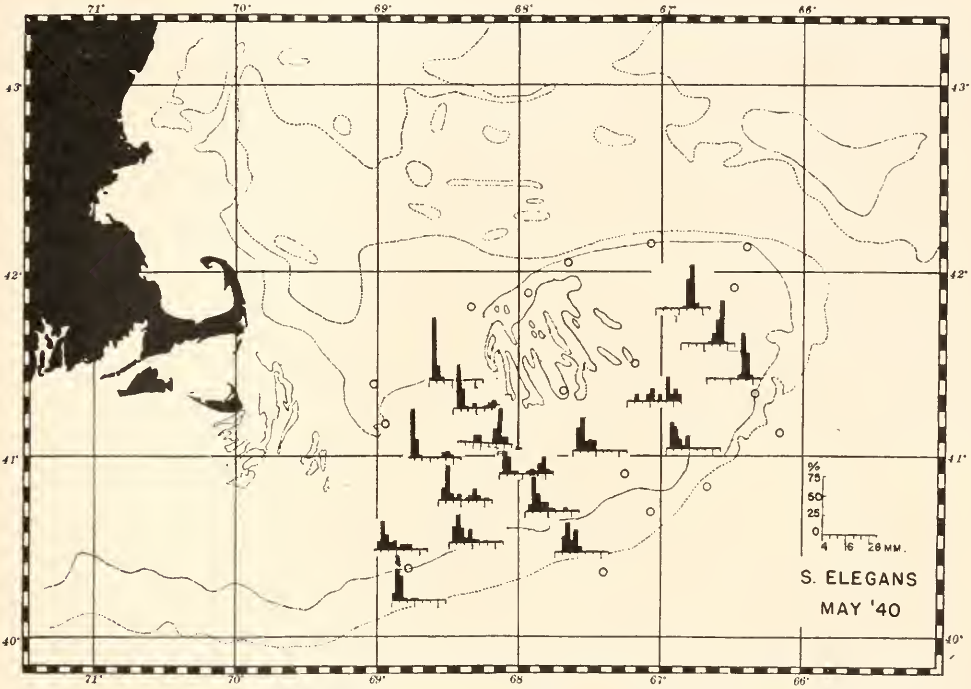


FIGURE 8. Length frequency distribution for individual stations in cruise of May 1940. Length measurements of *S. elegans* on percentage basis for individual stations, where numbers were sufficiently great.

From a study of the sequence of changes in the length and stage of maturity, as presented in Figure 9, information may be derived on the seasons of growth and reproduction of *Sagitta elegans* on Georges Bank. In the cruise of September 1939, the immature individuals were somewhat more numerous than either of the other stages, but both Stages II and III were well represented. The latter stages were much smaller in size than the corresponding groups taken during the spring months. The modal length of the mature Sagitta was only 16 mm. in September as compared to 23 mm. in the following March. In the January cruise Stages I and II were encountered in about equal numbers, but very few mature specimens were present. By March and April the majority of individuals had matured to the Stage III condition, and remnants of these animals were still found in diminishing numbers (and in smaller sizes) in the May and early

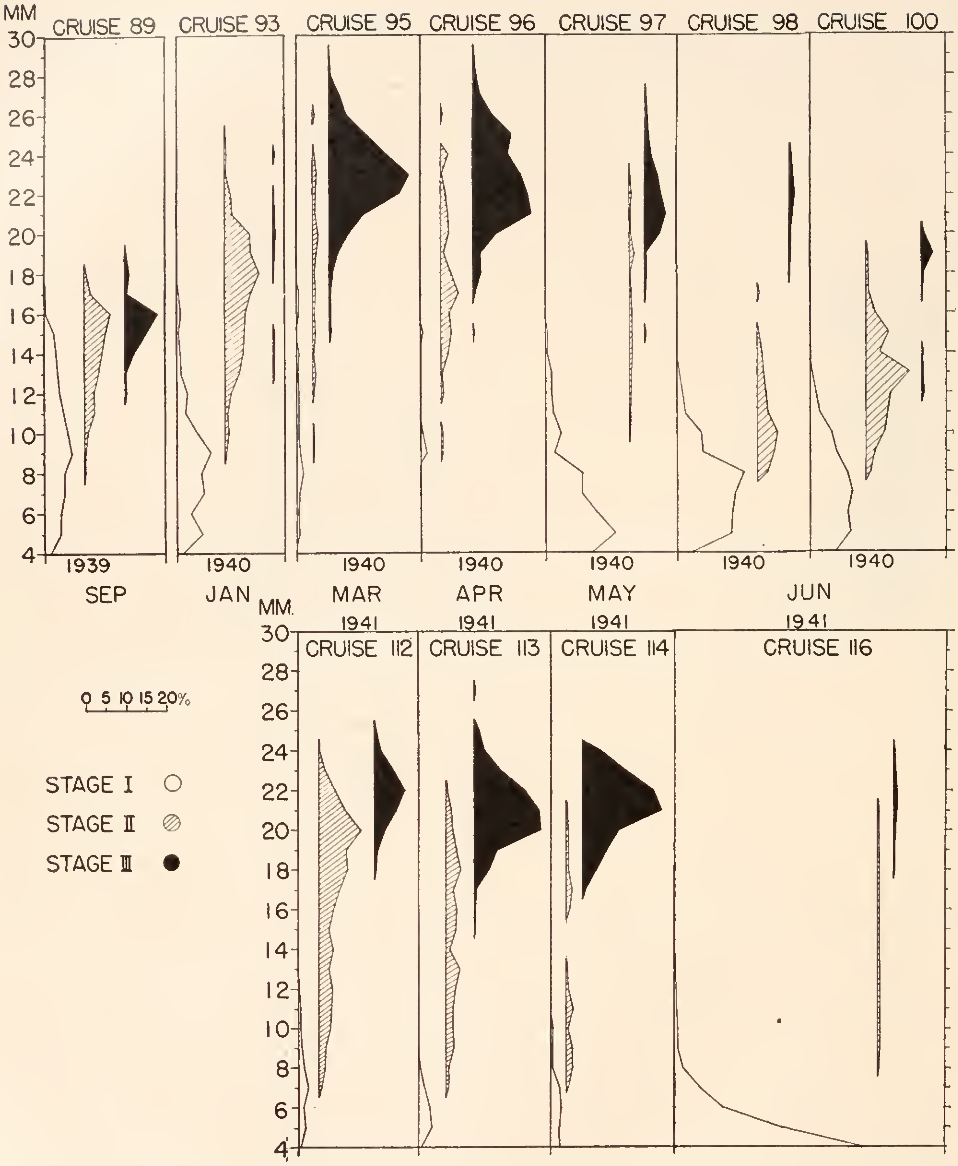


FIGURE 9. Stages of maturity and length frequency distribution for all cruises. Average values for *S. elegans* for all hauls in each cruise. Horizontal scale gives the percentage at each length, subdivided into the stages of maturity as indicated by the shading. Stage I—Immature, Stage II—Intermediate, Stage III—Mature.

June cruises. In May, however, the bulk of the catch consisted of immature specimens, and in the June cruises Stage I was also relatively the most abundant. Stage II was very scarce in May but appeared in increasing numbers (and in increasing sizes) in June. It is clear that this period of relative abundance of immature animals corresponds to the time of great increase in the actual numbers of smaller sizes which was noted in the previous section.

The striking difference observed in the lengths of the mature Sagitta at various seasons of the year is correlated in a general way with temperature. The shortest modal length for Stage III (16 mm.) occurred in September when the temperature of the water in the Mixed Area surpassed 16° C., the highest for the year, and the greatest modal length (23 mm.) was observed in March when the temperature reached a minimum of about 2.5° C. (Table II). As temperature increased through June, the mature sagittae became shorter again. Russell (1932) reported similarly that an inverse relationship existed between the length of the mature *Sagitta elegans* and the temperature of the water in the Plymouth Area, and the same tendency was observed in the Irish Sea by Pierce (1941). The maximum temperature recorded by Russell was about the same as in the present investigation, but the average length of his Stage III animals was only about 10 mm. On the other hand, the minimum temperature off Plymouth did not fall below 8° C. and the average length of the adult Sagitta was about 12 mm. in February, about 16 mm. in April to May 1931, and about 20 mm. in May 1930. No animals of length greater than 22 mm. were taken by Russell, whereas specimens as long as 30 mm. were encountered on Georges Bank. Our observations therefore agree with Russell's in revealing an inverse relation between temperature and body length, but the actual values are quite different. We have no information at present as to the mechanisms which underlie these relationships.

The changes in the Sagitta population during the spring of 1941 as revealed by the four corresponding cruises of that year, agree in general with those of the previous spring. There is, however, consistent indication that growth and reproduction were delayed in 1941 (Figure 9, bottom), although no important difference in the temperature of the Mixed Area water occurred for the corresponding months (Table II). In March 1941, there were relatively many more Stage II animals and many fewer Stage III individuals than in March 1940. The mature Sagitta dominated the scene in April 1941 and persisted in much higher relative abundance in May of that year than had been the case in 1940. At the same time it is to be noted that no important increase in Stage I is indicated in May 1941, in marked contrast to the previous year. Nevertheless, by early June 1941 (Cruise 116) small-sized Stage I animals appeared in very great numbers both relatively and absolutely. Stage III was reduced to a small remnant at this time, and there was no sign of an increase in the numerical strength of Stage II as had occurred in the previous June. It may therefore be concluded that in 1941 both the appearance of mature adults and the production of the large spring crop of young took place about a month later than in 1940.

An attempt may be made from the foregoing information to ascertain the annual cycle of growth and reproduction of *Sagitta elegans* on Georges Bank, but it is obvious that the gaps in the record during months in which no data could be obtained prevent final conclusions from being reached. It seems almost certain, however, that the chief period of reproduction centers in April or May because of the very high proportion of mature adults in those months, and the appearance subsequently of very large numbers of small, immature individuals. The adults which produced these animals apparently die off in June and the new crop of Sagitta matures during the summer to form a distinct generation of adults. This supposition is strongly supported by the fact that the Stage III animals found in September were of an entirely different size from those which had been prevalent in the spring. The graph (Fig. 9) suggests that the spring crop of young animals begins developing into Stage II in June with the possible appearance of a few of the new small-sized adults. The Stage I animals present in September may represent either the end of the spring and summer spawning or the beginning of the reproductive activity of the new generation of small adults. By January practically all of these small Stage III animals have disappeared and the immature individuals present are presumably their progeny. It seems safe to assume that the latter then slowly mature to produce the relatively large group of adults found in March and April, thus completing the cycle.

On the basis of the foregoing reasoning we may tentatively conclude that on Georges Bank *Sagitta elegans* undergoes one major period of reproduction during the spring months and that a distinct second generation is produced sometime in the late summer or autumn. It must be borne in mind, however, that during July and August and in the period between September and January, when no observations were made, another complete generation could have been formed. Russell (1932) believes that *Sagitta elegans* may complete a generation in as little as 43 days during the warmer months; and he has interpreted his data from the Plymouth Area as indicating that this species produces four or five generations during the course of the year. Pierce (1941), however, concludes that in the Irish Sea there is but one chief spawning period for *S. elegans* annually, extending from January through May. Our present data definitely indicate the existence of one major and one minor generation of this species in the Georges Bank area, but do not justify as yet any assumption that further generations occurred during the year.

Discussion

Information derived from the foregoing analyses of the distribution, growth, and reproduction of *Sagitta elegans* may now be examined as a contribution to the ecology of this species on Georges Bank particularly with reference to the currents of the region. Our previous knowledge of the occurrence of *S. elegans* off the New England coast has been summarized by Redfield and Beale (1940) in relation to their own studies of the sagittae in the Gulf of Maine. Although no special study of Georges Bank was undertaken by these authors, occasional stations on the Bank were occupied in the course of their survey, and at these stations large numbers of *S. elegans* were almost always encountered. These rich hauls contrasted sharply with the situation in the central area of the Gulf of Maine where the species was very scarce at practically every station. The explanation offered by Redfield and Beale is that water barren in respect to *Sagitta elegans* enters the Gulf each year and circulates through the central area so rapidly that sufficient time does not exist for large populations of this species to build up, even though the ecological conditions may be favorable in other respects. On Georges Bank, in contrast, these authors suggest that the water mass is sufficiently permanent to allow sagittae to accumulate and to further augment their number through effective reproduction.

The present investigation, based as it is on a much larger number of hauls on Georges Bank itself, not only tends to confirm the general suggestions of Redfield and Beale, but also provides strong evidence on the degree of permanence of the bank water. On the other hand, scrutiny of all the present data indicates that ecological factors other than simple transportation and accumulation play important roles in determining the distribution and abundance of the plankton. Redfield and Beale themselves point out that the central area of the Gulf of Maine supports a rich endemic population of Crustacea in spite of the fact that these animals are presumably subject to the same dislocating action of the currents as was invoked to explain the scarcity of *S. elegans* in that area.

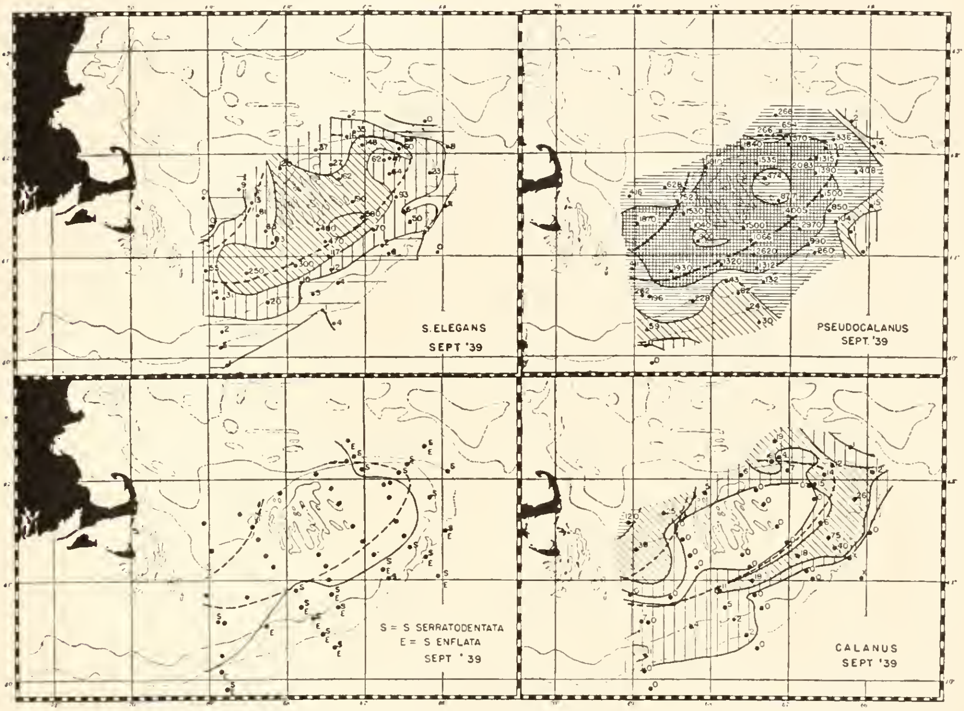


FIGURE 10. Comparison of the horizontal distribution of certain species of plankton in September 1939 (cruise 89). Distribution of *Sagitta elegans* is compared with that of *S. serratodentata*, *S. enflata*, *Pseudocalanus minutus*, and *Calanus finmarchicus*. Values shown are average numbers per cubic meter (per 10 cubic meters for *S. elegans*) for the whole water column for all stages of each species. Plankton Sampler hauls. Occurrence records for *S. serratodentata* and *S. elegans* are from the 75 cm. silk net hauls. The boundary of the Mixed Area is indicated by the heavy broken line. The symbol "X" represents a value of less than one individual per unit volume.

The agreement of the distribution of *S. elegans* and of certain other species of plankton with the disposition of the water masses on Georges Bank during the present survey is well illustrated by the comparison for the cruise of September 1939, presented in Figure 10. Allusion has been made above to the close conformity of the main abundance of *Sagitta elegans* to the limits of the Mixed Area for that period. Two other species of *Sagitta* appeared during the September cruise in sufficient abundance to permit significant analysis of distribution to be made, although numbers were so small that presence or absence alone has been indicated in the chart of their occurrence (Figure 10, lower left). It is seen that the distribution of *S. serratodentata* and *S. enflata* was confined to the southern and eastern margins of the Bank in distinct contrast to *S. elegans*. A remarkably close agreement is observed between the line limiting the occurrence of the former and the margin of the Mixed Area. *S. serratodentata* occurred farther up on the Bank than did *S. enflata* but neither had been carried into the Mixed Area at more than one or two points. This fact shows that a very small amount of water, if any, was entering the Mixed Area from the south or the east at the time of this cruise.

It is of value to compare this striking case of the separation of two morphologically similar species by the hydrographical condition on the Bank with an equally clear-cut reciprocity in the distribution of two copepods, *Pseudocalanus minutus* and *Calanus finmarchicus* (Figure 10, upper right and lower right). Pseudocalanus occurred at all stations on the Bank during this cruise in very large numbers except along the southern and northeastern margins, but concentrations of over 1000 individuals per cubic meter were limited to the central region and to the north central margin, this zone of abundance corresponding closely to the Mixed Area.

In the case of Calanus, on the other hand, no specimens whatsoever were taken in the central portion of the Bank and very low counts were obtained for this species at every station within the Mixed Area (Fig. 10). Similarly small catches of Calanus were made along the extreme southern margin of the Bank and to the northeast, but considerable numbers were taken at the stations to the north and west toward the Gulf of Maine, and in the zone between the Mixed Area and the southern margin of the Bank. The tongue of water, rich in Calanus, which appears extending across the eastern end of the Bank and curving south and then west, is a clear indication that at the time of this cruise a current carrying Calanus from the Gulf of Maine was flowing around the margin of the Mixed Area and forming a wedge between it and the water masses to the south. At the same time mixed water, barren in respect to Calanus, from the central eddy of the Mixed Area appears to have been draining off to the west down the middle of the Bank. *Calanus finmarchicus* therefore appears to be a species which can endure neither the homogeneous water of the Mixed Area nor the warm, saline conditions to the south, but which thrives in the water of the Gulf of Maine. Thus Calanus is similar to *S. serratodentata* and *S. enflata* in being sharply excluded from the Mixed Area of Georges Bank, whereas Pseudocalanus and *S. elegans* are chiefly abundant within it.

Although one could argue that even if *S. serratodentata* and *S. enflata* found their way on to Georges Bank, they could not survive there because of lower temperatures and salinities, it is impossible to invoke these factors as preventing the occurrence of *Calanus finmarchicus* in the central part of the Bank. As we have seen, the water of the Mixed Area is chiefly derived from the Gulf of Maine where Calanus is abundant, and the temperature and salinity values of this water are generally intermediate between those of the upper and lower strata of the Gulf. Some other factor must be found which could prevent the reproduction of Calanus in the Mixed Area or which could cause its destruction (or both), and which does not affect Pseudocalanus adversely. Perhaps sediment resulting from the turbulence in the Mixed Area is harmful to Calanus or possibly some essential element is lacking, such as accessible bottom water of low temperature (cf. Bigelow and Sears, 1939), or a necessary food organism. On the other hand, destruction of Calanus might be brought about chiefly by a predator, and as such *S. elegans* suggests itself for future study since it populates so abundantly the very area from which Calanus is removed.

Since other ecological influences besides the purely mechanical action of the current system are apparently controlling the occurrence of Calanus on Georges Bank, it seems probable that additional, as yet undetermined, factors may be important in accounting for the special richness of the population of *Sagitta elegans* in the Mixed Area. It now appears well established that the relative permanency of the bank waters makes possible the maintenance of an adequate breeding stock of *S. elegans* from one period of reproduction to another, but, in addition the data presented above indicate that the breeding and growth of this species is especially successful in the mixed bank water during the spring months, and perhaps at other seasons as well.

This suggestion that the great abundance of *Sagitta elegans* within the Mixed Area of Georges Bank is due not merely to mechanical accumulation but also to especially favorable local conditions, is supported by observations from other regions. Bigelow and Sears (1939) consider that this species is regularly endemic in the waters overlying the inner half of the continental shelf from Cape Cod south possibly to the offing of Chesapeake Bay. There are no large, permanent eddies in this area. Russell (1939) pointed out that *S. elegans* occurred in dominant numbers around the British Isles in areas where *mixed oceanic and coastal water* occurred. This author does not report any accumulation of the species in these areas through a mechanical action of the currents, but states that "The incursion of oceanic water gives rise to conditions in which a different plankton community, typified by *Sagitta elegans*, flourishes."

It is obviously of importance to inquire what characteristic of the mixed water of Georges Bank may make it an especially favorable environment for the reproduction and growth of *Sagitta elegans*. Temperature and salinity may apparently be ruled out since we have seen that the values for these factors are within the range of those found in the central region of the Gulf of Maine where this species is relatively scarce. Three other possibilities suggest themselves: (1) the turbulence and the attendant vertical homogeneity of the water, (2) the shallowness of the water, and (3) the existence of some element in the water originally derived from the shore or from the bottom. Although we do not have the means at present to decide between these, or other possibilities for Georges Bank, some evidence may be obtained from the ecological conditions of other regions of abundance for this species. Such comparison appears to eliminate vertical mixing of the water, *per se*, as particularly favorable since this condition does not characterize most of the other areas of occurrence of *S. elegans*. The waters of the continental shelf to the south, and of Massachusetts Bay to the north, are both definitely stratified during the warmer portion of the year. Furthermore, breeding was found to be unsuccessful in the Bay of Fundy (Huntsman and Reid, 1921) where vertical mixing is strong.

The shallowness of the water might be regarded as a beneficial condition for *S. elegans* since all observations agree that this species occurs chiefly in the shoal water of the continental shelf or of off-shore banks both along the North American coast and around the British Isles. It is doubted, however, whether the nearness of the bottom could be a *direct*, favorable influence (a) because there is no evidence that this species has any ecological dependence on the bottom, (b) because its distribution on Georges Bank showed no relation to the depth contours, and (c) because in other regions it has been found to be as abundant in water 100 m. to 200 m. deep as in shallower zones.

The third suggestion, that some beneficial derivative of the shore or the bottom occurs in the shallower water of the coastal areas or the banks, remains as a possible, though vague explanation. Fraser (1939) remarks with reference to Sagitta that plankton in general can withstand "fairly big" physical changes and hence the very distinct separation in the distribution of species means some biological change in the water masses. The very definite tendency for abundant populations of *S. elegans* to occur in shoal areas therefore suggests the presence in the water of some chemical element derived from the shore or bottom or some food organism dependent on the bottom, which does not exist in the water of deeper regions.

We conclude, therefore, that *Sagitta elegans* is chiefly abundant within the Mixed Area of Georges Bank, first, because the relative permanence of this water mass allows it to accumulate there; second, because water of harmfully high temperature and salinity from the south is excluded; and third, because some indirect influence from the shore or bottom, absent in the deeper water to the north, favors its reproduction and growth. It remains for the future to re-examine the water of the Mixed Area in order to ascertain what conditions of feeding or other circumstance, render this water particularly favorable for *S. elegans* and certain other types of plankton, and particularly unfavorable for other, closely related species.

Finally, it may be emphasized that the observations from the eleven cruises of this investigation have shown that Georges Bank supports a relatively abundant population of *S. elegans* throughout the year, and that the center of concentration of Sagitta was always found within the Mixed Area. Furthermore, the contours of the Sagitta population were shown to conform in general to the limits of the Mixed Area. Frequently this rich area was *completely surrounded* by water in which *S. elegans* was very scarce or absent. It appears then that this species is an adequate indicator for the presence of Mixed Area water. We know, as a result, that the eddy of homogeneous water on Georges Bank may be regarded as permanent to the extent to which the population of *Sagitta elegans* has been shown to maintain its integrity there from season to season. It is of special interest to note that a nucleus of this species apparently retained its position on the Bank throughout the winter, during the period when the breakdown of stratification in the surrounding areas might be expected to make possible a flow of Gulf water directly across the Bank. The bubble of mixed water on the Bank therefore either fails to be dislodged by hydrographic forces, or is renewed so slowly that the population of Sagitta is able to maintain itself within the Mixed Area despite the water movement. By similar application of these findings, it should be feasible to employ *S. elegans* as an indicator to trace the movements of Mixed Area water in future studies of the ecology of the region.

SUMMARY

1. The quantitative distribution, size, and stages of maturity of *Sagitta elegans* in the waters of Georges Bank have been determined from plankton hauls made on a network of stations occupied during 11 cruises from September 1939, to June 1941.

2. The area of relatively homogeneous water overlying the central portion of Georges Bank was found to change in extent from cruise to cruise, but to be sharply delineated from the surrounding stratified water masses, and has been designated as the "Mixed Area."

3. The abundance of *S. elegans* varied in individual hauls from a maximum of 165 specimens per cubic meter to zero, but averaged more than 10/m.^{3} at stations within the Mixed Area. The deeper hauls were numerically richer than the shallow hauls at more than half the stations. The existence of a diurnal vertical migration was revealed in certain cases, but varied greatly from cruise to cruise.

4. In horizontal distribution the greatest concentrations of *S. elegans* were found within the central homogeneous water mass of the Bank and a close agreement was disclosed between the contours of abundance and the boundary line of the Mixed Area. A center of abundance for this species existed on the Bank throughout the year.

5. The modal length of the mature sagittae increased from 16 mm. in September, when the water temperature was the highest, to 23 mm. in March, when the temperature was at the minimum for the year. Some specimens as long as 30 mm. were encountered.

6. The chief period of reproduction for *S. elegans* on Georges Bank centered in April in 1940 and in May in 1941. Following these periods very large numbers of small, immature individuals appeared. Evidence is presented that a distinct, second generation was produced during the late summer or autumn.

7. *S. serratodentata* and *S. enflata*, which were taken chiefly in the September cruise, occurred entirely outside the margin of the Mixed Area. Comparison is made with a similar case of reciprocal distribution found for two species of copepods: *Calanus finmarchicus*, which was excluded from the Mixed Area, and *Pseudocalanus minutus*, which was chiefly abundant within the Area.

8. Our observations support the suggestion of Redfield and Beale that *S. elegans* tends to accumulate on Georges Bank because of the relative absence of dislocating currents. However, reasons are advanced for believing that other characteristics of the Mixed Area water should be scrutinized as being particularly favorable for the growth and reproduction of *S. elegans*, and particularly unfavorable for the existence of other species of plankton.

9. The persistence of the population of *S. elegans* on Georges Bank throughout the year is a valuable index of the degree of permanence of the homogeneous bank water. The species recommends itself as an indicator for tracing movements of the Mixed Area water in future studies.

LITERATURE CITED

Bigelow, H. B., and M. Sears, 1939. Studies of the waters of the continental shelf, Cape Cod to Chesapeake Bay. III. A volumetric study of the zooplankton. *Memoirs of the Mus. Comp. Zool. at Harvard College*, **54**: 189–373.

Clarke, G. L., 1934. Factors affecting the vertical distribution of copepods. *Ecological Monographs*, **4**: 530–540.

Clarke, G. L., and D. F. Bumpus, 1940. The Plankton Sampler—an instrument for quantitative plankton investigations. *Linnological Society of America, Special Pub.*, (No. 5): 1–8.

Fraser, J. H., 1939. The distribution of Chaetognatha in Scottish waters in 1937. *Jour. du Conseil*, **14**: 25–34.

Huntsman, A. G., and M. E. Reid, 1921. The success of reproduction in Sagitta elegans in the Bay of Fundy and the Gulf of St. Lawrence. *Trans. Roy. Canadian Inst.*, **13**: 99–112.

Iselin, C. O'D., 1939. Some physical factors which may influence the productivity of New England's coastal waters. *Sears Found. Jour. Mar. Res.*, **2**: 74–85.

Pierce, E. L., 1941. The occurrence and breeding of Sagitta elegans Verrill and Sagitta setosa J. Müller in parts of the Irish Sea. *Jour. Marine Biol. Assoc.*, **25**: 113–124.

Redfield, A. C., and Alice Beale, 1940. Factors determining the distribution of populations of Chaetognaths in the Gulf of Maine. *Biol. Bull.*, **79**: 459–487.

Riley, Gordon A., 1941. Plankton studies. IV. Georges Bank. *Bull. Bingham Oceanographic Coll.*, **VII**: Art. 4, 1–73.

Riley, Gordon A., 1942. The relationship of vertical turbulence and spring diatom flowerings. *Sears Found. Jour. Mar. Res.*, **5**: 67–87.

Sears, Mary, 1941. Notes on the phytoplankton on Georges Bank in 1940. *Sears Found. Jour. Mar. Res.*, **4**: 247–257.

Russell, F. S., 1932. On the biology of Sagitta. The breeding and growth of Sagitta elegans Verrill in the Plymouth area, 1930–31. *Jour. Mar. Biol. Assoc.*, **18**: 131–146.

Russell, F. S., 1933. On the biology of Sagitta. IV. Observations on the natural history of Sagitta elegans Verrill and Sagitta setosa J. Müller in the Plymouth area. *Jour. Mar. Biol. Assoc.*, **18**: 559–574.

Russell, F. S., 1939. Hydrographical and biological conditions in the North Sea as indicated by plankton organisms. *Jour. du Conseil Intern. pour l'Expl. de la Mer*, **14**: 171–192.

THE MORPHOLOGY AND LIFE HISTORY OF THE DIGENETIC TREMATODE, ZOÖGONOIDES LAEVIS LINTON, 1940

HORACE W. STUNKARD

(*New York University*)

Linton (1940) described the adult stage of *Zoögonoides laevis* and distinguished between this species and *Z. viviparus* (Olsson, 1868) Odhner, 1902, the type and only other known representative of the genus. He found the worms in the intestine of *Tautoga onitis* and an immature specimen from the round herring, "*Etrumens sadina*" (= *Etrumeus teres*) was referred provisionally to *Z. laevis*.

During the summer of 1942, tailless cercariae were found emerging from *Columbella* (= *Mitrella* Rizzo) *lunata* collected in the Woods Hole region. Their striking resemblance to the cercariae of *Zoögonus lasius* (Leidy, 1891) Stunkard, 1940 indicated that the two were closely related. Furthermore, their morphological agreement with *Zoögonoides laevis* suggested that they might be larvae of the latter species. Experiments demonstrated the correctness of the hypothesis and the successive stages in the life cycle have been obtained. The cercariae develop in sporocysts in the lymph spaces of *C. lunata*, penetrate into *Nereis virens* where they become metacercariae, and sexual maturity is attained in the intestine of *T. onitis*. The eggs are large, without shells, and contain active, ciliated miracidia when extruded. The larvae hatch in sea water and invade the snails where the asexual generations are produced. The life history was reported in abstract (Stunkard, 1942).

EXPERIMENTS

The methods employed in the present study were similar to those described by Stunkard (1938, 1941) in reports on the life history of Zoögonus. Over 2000 specimens of *Columbella lunata* were collected from algae taken at several locations. They were isolated in groups of ten or more in large stender dishes and observed morning and evening for five days to obtain preliminary information concerning the incidence of infection and also to obtain infective cercariae for life history experiments. Water in the dishes was changed twice daily and bits of algae were provided as food for the snails. When cercariae appeared in a dish, the snails were isolated individually to obtain the one or more which harbored the parasite. Of the snails from which no larvae emerged, several hundred were crushed and examined to secure more complete information concerning the total incidence of infection. In different collections the total infection varied from 0.5 to 5 per cent, although about one-fourth of the infected snails did not liberate cercariae. The snails are small and the cercariae are relatively large; consequently the number of cercariae which emerged from any snail was small, usually two to five in 24 hours. Sometimes an infected snail would not liberate cercariae for three or four days. The larvae are hardy and may live for four days in sea water. In form and behavior, they resemble those of *Zoögonus lasius*.

They emerge during the day and at night. The posterior end of the body exudes a sticky substance and bits of debris adhere to it. The secretion causes the cercariae to stick to the wall of a pipette and it is difficult to transfer them from one dish to another. They adhere to the substratum or move about by alternate attachment of the ends of the body, using the oral sucker to attach the anterior end. Ordinarily the larvae do not encyst in fresh sea water, but occasionally one would extrude cystogenous material on a slide if the water evaporated enough to seriously disturb the osmotic equilibrium. The cystogenous secretion is often emitted when the larvae are placed in solutions of vital dyes, and sometimes encystment is complete. If sporocysts containing fully grown cercariae are removed from a snail and left for several hours in sea water, and especially if the sporocysts become moribund, the older cercariae may encyst in them. Also, sporocysts removed from dead snails may contain encysted cercariae.

Infection of Second Intermediate and Final Hosts

Since the cercariae are unable to swim, and since they do not normally encyst in sea water, it was apparent that the next host must be a bottom-dwelling animal. Accordingly, various molluscs, crustaceans and worms were placed in finger bowls with normally emerged larvae. The larvae disappeared in a few hours from the dish with *Nereis virens*, whereas they persisted for at least two days in dishes with the other animals tested. A parapodium was cut from a living specimen of Nereis and placed in a stender dish with four cercariae; four hours later, three of them had penetrated into the parapodium and two had encysted. These observations indicated that Nereis may serve as an intermediate host, although they do not preclude the possibility that other animals also may be utilized in nature. When it was observed that the larvae would encyst in Nereis, mass infection was attempted. Sixteen infected *C. lunata* and four Nereis were placed in a small aquarium provided with about 2 cm. of sand on the bottom and with a cheesecloth cover to permit the entrance of sea water and to prevent the escape of the snails and worms. These worms were exposed to cercariae for two weeks and then fed to tautogs which had been isolated without food for seven weeks. The fishes were dissected three days later and dozens of young *Z. laevis*, some of them hardly larger than cercariae, were recovered. In addition, there were other, larger and more mature specimens of *Z. laevis* which undoubtedly represented a natural infection, acquired by the fishes before they were caught. Tautogs which were dissected immediately after capture were always infected; the number of worms taken from the intestine varied from a few to about three hundred, and in heavy infections all stages of development from recently excysted metacercariae to gravid specimens were found. The small worms of natural infection were morphologically indistinguishable from those of the experimental infection. It is apparent from these experiments that the cercariae from *C. lunata* encysted in Nereis, that they later became established in the intestine of the tautog, and that they are larvae of *Z. laevis*. Furthermore, it is apparent that the metacercariae are infective for the final host soon after encystment.

Infection of the First Intermediate Host

Gravid worms from natural infections often contain many active, ciliated miracidia, enclosed in membranous capsules. Since the worms have small, deficient vitellaria, the eggs lack shells. When such gravid specimens were compressed under a coverglass for study or for fixation, occasionally the egg nearest the genital pore was expelled. In a few instances, after the addition of tap water, the membrane ruptured and the larva emerged and swam about. Dilution of the sea water is not necessary, however, to induce hatching of the miracidia. When mature worms are removed from the fish to sea water, they usually extrude eggs and the miracidia emerge in a few hours. The larvae elongate in swimming, rotate on the long axis, and progress rapidly in a spiral path. They become uniformly distributed in the water and no obvious reaction to light was observed. Since the miracidia emerge from the eggs and swim vigorously, it is apparent that they penetrate the snail host to establish the infection. Normally emerged, free-swimming miracidia were not noticeably attracted toward specimens of *C. lunata* placed in a dish with them. It appears that their contact with snails is accidental and larvae would sometimes make contact with a snail and then swim away. Penetration by the larvae was not observed and it seems probable that they enter the branchial cavity of the snail with water and then invade the tissue. This hypothesis is supported by the location of primary sporocysts dissected from experimentally infected snails.

To obtain experimental infection of *C. lunata*, 50 snails, which had been isolated for two weeks without the appearance of any cercariae, were placed in a small tank with two tautogs and left there for 19 days. At the end of that time 30 of them were recovered and transferred to finger bowls. The fishes were dissected and more than two hundred mature specimens of *Z. laevis* were taken from them. Five of the snails which had been exposed to infection were dissected and young sporocysts were found in two of them. The parasites, undoubtedly primary sporocysts, were small, oval to irregularly shaped sacs containing groups of germinal cells. The other snails were kept in a finger bowl and those that died or became weak were crushed and examined. Sporocysts which contained unidentifiable germ masses were observed in the snail tissues and at the end of the season, seven weeks after the snails had been placed in the tank with the fishes, the twelve remaining snails were killed and examined. Ten of them contained sporocysts; six were heavily and four were lightly infected. Each of the heavily infected snails contained hundreds of sporocysts; each of the lightly infected ones contained fifty to one hundred sporocysts. The number of sporocysts suggests that they were the second or daughter generation. Several of the sporocysts contained large, elongate germ balls but differentiation had not proceeded to a point where they could be recognized as either young sporocysts or cercariae. The snails had been examined daily for emerged cercariae and since none were observed, it is evident that the infection of the snails was an experimental one. The cercariae are produced in sporocysts, but the number of sporocyst generations and the time from entrance of miracidium to emergence of cercariae is yet unknown. Due to the slow development of the parasites and the shortness of the working season, it was impossible to complete the cycle in experimentally infected hosts, but experimental infection of the definitive and both intermediate hosts was secured and, accordingly, the data appear to provide convincing proof of the life cycle.

Both adults and larvae of *Z. laevis* were studied alive, with and without vital staining and after fixation and staining. Specimens were fixed in the extended condition under coverglass pressure for whole mounts and others without pressure for sections, in order that distortion from compression could be recognized.

Descriptions

The description of the sexually mature stage of *Z. laevis* given by Linton (1940), although limited to gross morphology, is adequate for identification of the species. Since the specimens of the present study agree substantially with the account of Linton and are from the same host and the same locality, their identity with those of Linton is assumed.

The Adult

The worms are pale yellow to reddish in color and the larger ones are conspicuous on the opened intestine of the fish. They occur throughout the length of the intestine, although the majority are located in the posterior portion. Often they are deep between the villi and they may adhere tenaciously, especially with the powerful acetabular sucker. On removal, they frequently pull away the portion of the intestine grasped in the acetabulum (Fig. 1). In sea water they are relatively sluggish and tend to remain in a contracted condition. The body is very muscular and the shape varies with the degree of extension. Typically the worms are terete, fusiform, with the posterior half of gravid specimens saccate and distended by many uterine coils. The preacetabular region is more active and when extended it tends to bend ventrally forming a shallow ventral concavity.

Gravid specimens (Fig. 2) fixed under a coverglass, stained and mounted, vary from 0.6 to 1.1 mm. in length and 0.2 to 0.45 mm. in width. For well extended specimens, it is necessary to compress the worms, which increases their width. The acetabulum is slightly anterior to the middle of the body and measures from 0.195 to 0.27 mm. in diameter. As noted by Linton, its aperture is transverse, but he made no mention of the peculiar muscular development which determines the shape. The opening of the sucker is provided with a powerful sphincter which, when contracted, forms thickened muscular masses (Figs. 1, 2) anterior and posterior to the aperture. These masses may protrude

---

Explanation of Plate

Abbreviations

am	anterior germinal mass	ov	ovary
cs	cirrus sac	pd	penetration gland ducts
ed	excretory duct	pg	penetration gland
em	embryo	pm	posterior germinal mass
ev	excretory vesicle	sr	seminal receptacle
gd	gland duct	ts	testis
gp	genital pore	ut	uterus
in	intestine	vg	vitelline gland

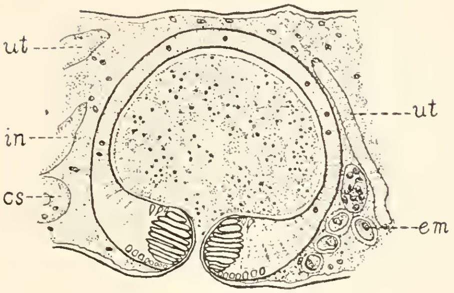
All figures are of *Z. laevis*

FIGURE 1. Sagittal section through the acetabulum of adult, showing the sphincter and lumen filled with tissue from intestine of the host; anterior end at left of the figure.

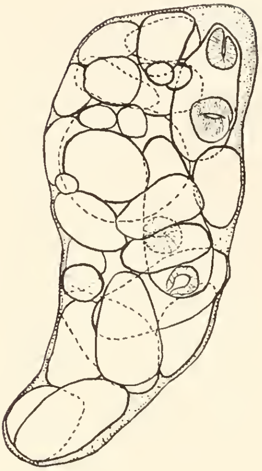
FIGURE 2. Adult, whole mount, flattened under coverglass, stained and mounted; dorsal view.

FIGURE 3. Sporocyst from *Columbella lunata*, natural infection, with developing cercariae.

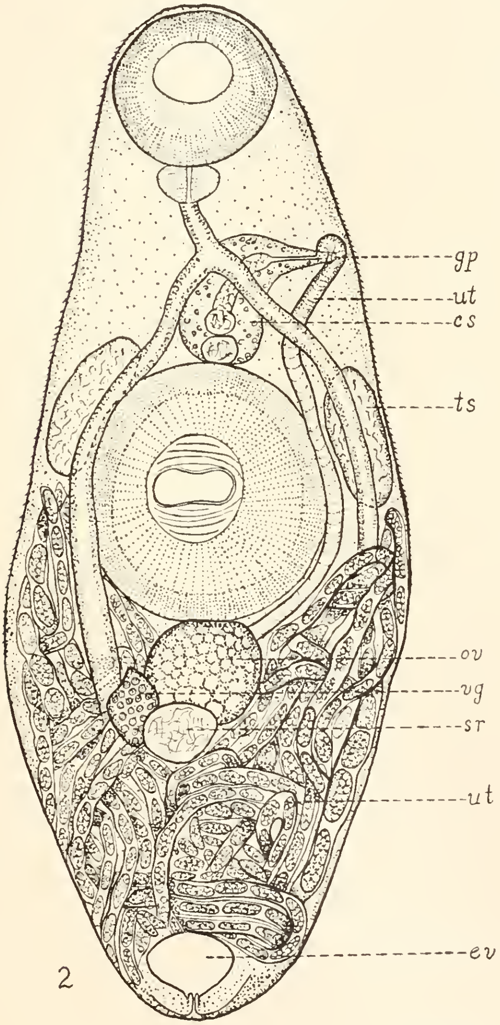
FIGURE 4. Cercaria, composite drawing from free-hand sketches of living larvae, showing details of structure.


ut
ut
in
cs
em

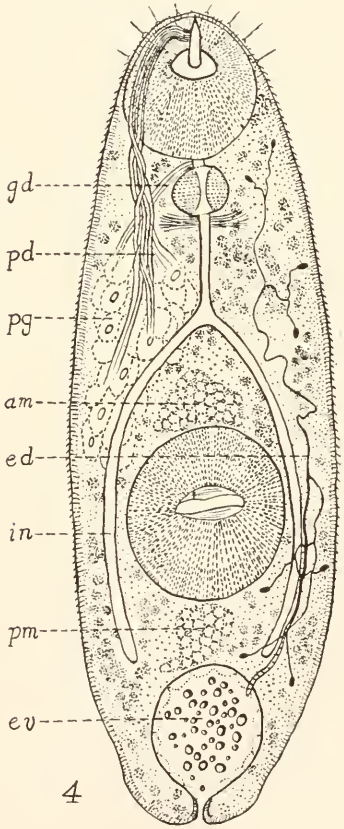
1



3


gp
ut
cs
ts
ov
vg
sr
ut
ev

2


gd
pd
pg
am
ed
in
pm
ev

4

PLATE I

into the lumen, giving the opening of the sucker a dumb-bell shape. The cuticula is thick and in living specimens shows reticulate furrows. Minute, straight, retrose spines are embedded in it. They measure about 0.006 mm. in length; their bases are adjacent to the basement membrane and their tips barely protrude from the surface of the cuticula. The spines are seldom visible on stained and cleared specimens but show well in sections stained with iron haematoxylin. They are somewhat smaller in the area around the mouth and become sparse on the posterior part of the body. There are small papillae at the anterior end; presumably these are the structures which in the cercaria bear sensory bristles.

The oral sucker measures from 0.1 to 0.145 mm. in diameter, with the mouth subterminal in position. There is a short prepharynx and the commissure of the nervous system, although morphologically prepharyngeal, usually appears to lie above the pharynx. The pharynx measures 0.03 to 0.06 mm. in diameter and the esophagus extends posteriad about one-half the distance to the acetabulum where it joins the intestinal ceca. The ceca pass backward on the dorsal side of the body above the cirrus sac, metraterm and testes, and end just behind the level of the seminal receptacle. They have a narrow and almost uniform diameter.

The excretory system of the adult is morphologically identical, except for slight changes incident to growth and sexual maturity, with that of the cercaria (Fig. 4). The pore is terminal and a short canal leads from it to the bladder. The cuticula is thickened at the posterior end of the body and the circular muscles are modified to form sphincters at the outer and inner ends of the excretory canal. When the pore is closed the wall of the canal may lie in longitudinal folds which cause the lumen to be radiate in cross section. At the posterior end of the worm the longitudinal muscles of the body wall converge in bands and are inturned and inserted on the wall of the excretory canal. From this region other muscles continue meridionally in the wall of the excretory bladder, giving the rosette appearance depicted in the figure by Linton. It is the contraction of these muscles which expels the contents of the bladder. The bladder is saccate and when filled may extend one-third of the distance to the acetabulum. From the antero-lateral surface on each side, a common primary collecting duct passes forward in a sinuous course to the level of the acetabulum. Here it divides into anterior and posterior secondary branches. The anterior duct divides near the bifurcation of the alimentary tract into two tertiary ducts. One passes forward and at the level of the pharynx divides into two capillaries which lead to the anterior pair of flame cells; the other turns backward and divides into two capillaries that lead to a pair of preacetabular flame cells. The posterior secondary collecting duct divides in a pattern which is the counterpart of the anterior one. The flame cell formula therefore is $2[(2 + 2) + (2 + 2)]$. The location of the flame cells and tubules of the cercaria is shown in Figure 4 and the system persists with only minor changes, due to differential growth, in the adult condition. The wall of the excretory bladder contains circular as well as longitudinal fibers and the cells of the epithelial lining bear cilia or striated brush borders. The openings of the collecting ducts are surrounded by areas where the ciliary beat is conspicuous. The walls of the collecting ducts contain circular and longitudinal fibers and the lumen is ciliated; the ciliary beat is toward the bladder. Ciliated patches occur in other portions of the collecting system and the ciliated tufts of the flame cells measure 0.009 to 0.12 mm. in length.

The testes are ventral, situated at the sides of the acetabulum, although they may be displaced forward or backward until they lie anterior or posterior to the sucker. They are oval, compressed laterally and in gravid specimens measure from 0.12 to 0.17 mm. in length and 0.07 to 0.12 mm. in width. Vasa efferentia arise from the dorso-antero-median faces of the testes and unite to form a single duct which immediately enters the posterior end of the cirrus sac where it expands to form a bipartite, sigmoid seminal vesicle. The duct leading from the vesicle is surrounded by cells of the prostate and usually contains large secretory droplets. The cirrus is spined and although it was not observed in the extruded condition, the structure of the parts indicates that it is eversible. The cirrus sac is large and extends from the level of the acetabulum to the common genital pore located near the right ventral margin at the level of the bifurcation of the alimentary tract. The posterior end of the sac is dorsal in position and it curves anteriad, laterad and ventrad to the pore. The opening of the cirrus sac is below and slightly behind that of the metraterm.

The ovary is dorsal, immediately posterior to the acetabulum, either median or lateral, right or left. It is spherical to oval and in gravid specimens measures from 0.085 by 0.076 to 0.12 by 0.11 mm. The oviduct arises at the ventral, posterior margin of the ovary and turns dorsally under the anterior end of the seminal receptacle. Here it receives a very short duct from the receptacle. From the posterior side of this short duct, Laurer's canal passes posteriad and then dorsad by a sinuous course to open at the surface. The oviduct, after receiving the duct from the seminal receptacle, turns ventrad and toward the left where it expands and receives a short duct from the vitelline gland. The female duct then continues ventrad and posteriad forming the initial portion of the uterus. The uterus coils about and when filled with embryos, occupies the posterior half of the body. Its course becomes irregular and impossible to follow. The seminal receptacle is dorsal in position, immediately behind the ovary, with the anterior end of the vesicle above and partly overlapping the posterior end of the ovary. The vitelline gland is ventral, below and often slightly lateral to the seminal vesicle. Usually the anterior end of the vitelline gland is slightly anterior to the anterior end of the seminal vesicle. In the specimen shown in Figure 2, the structures are somewhat distorted by pressure exerted in flattening the worm. There is no "shell gland" and the miracidia develop in thin-walled, membranous sacs. The terminal portion of the uterus has strong muscle walls. It passes anteriorly below the ovary, turns dorsally at the right side of the cephalic portion of the ovary and continues across the right dorsal side of the acetabulum, passes below the cecum of the right side and turns ventrad above the terminal portion of the cirrus sac to open at the common genital pore. The development of the larvae in the uterus is similar to that in *Zoögonus lasius.*

The Miracidium

The uterus of *Z. laevis* is filled with developing miracidia and the terminal coils contain ciliated larvae. The fully formed miracidium is oval, pointed anteriorly and entirely covered with long, closely-set, powerful cilia. In the egg membrane, the cilia beat vigorously and the larva performs muscular movements;

the anterior tip is frequently protruded, turned about and then retracted. The anterior portion of the larva contains two gland cells with ducts which open at the tip; droplets were observed emerging from these openings. The nuclei in the anterior portion of the miracidium were vesicular and stained faintly; in the posterior portion there is a group of nuclei which stained deeply and which probably are those of germinal cells. There are two flame cells, one anterior and the other posterior in position, but the excretory ducts could not be followed. The number and arrangement of the ciliated epithelial cells were not determined; their nuclei are flattened and irregular in shape. The egg membrane is flexible and the shape varies with pressure; eggs in sea water and without a coverglass measured 0.076 to 0.08 mm. in length and 0.028 to 0.032 mm. in width. The miracidium is about 0.065 mm. long and 0.028 mm. wide and when the egg emerges from the worm into sea water the beat of the cilia is noticeably increased.

Sporocyst Generations

The miracidia penetrate into *C. lunata* and produce sporocysts but, as noted previously, the number of sporocyst generations in the life cycle of *Z. laevis* was not determined. Snails infected in the laboratory during the summer did not produce cercariae, but from these snails, exposed to miracidia for nineteen days and dissected six weeks later, large numbers of young sporocysts were recovered. When fixed and stained, they varied in size from 0.05 by 0.04 mm. to 0.144 by 0.11 mm., and most of them contained groups of germinal cells and germ balls, so immature and undifferentiated that it was quite impossible to determine whether they would become daughter sporocysts or cercariae. The young sporocysts were much smaller than cercariae. The smaller sporocysts were very numerous; in snails with a smaller number there was a corresponding increase in size. Whether all of these sporocysts belonged to a first daughter generation could not be determined. It is possible that some of the larger ones were first generation, i.e., primary sporocysts, and that some of the smallest ones were third generation. One of the larger ones contained five daughter sporocysts and 15 to 20 germ balls of varying sizes. Another, in addition to germ balls, contained two embryos that were as large as daughter sporocysts but were undifferentiated; they resembled developing cercariae but could not be identified positively. The sporocysts occupy the haemocoele of the snail.

In naturally infected snails, all of the sporocysts contained germ masses and developing cercariae; no sporocysts containing identifiable daughter sporocysts were observed. These sporocysts (Fig. 3) were oval to elongate, colorless sacs, with a birth pore at one end. When fixed and stained, they varied in size from 0.25 by 0.2 mm. to 0.86 by 0.32 mm. and usually contained several young cercariae in addition to masses of germinal tissue in various stages of development. All of the sporocysts were motile and the non-gravid ones were very active, elongating and shortening, bending and twisting. They changed from an oval to a cylindrical shape in which the length was as much as eight times the diameter. When elongate they would often bend in a C-shape and then thicken at one end, becoming clavate in form. In locomotion the anterior end is protruded as a long, slender process. It then begins to thicken near the tip until a bulbous enlargement is formed at or near the end. This enlargement increases in size as the contraction of circular and longitudinal muscles in the more posterior portion thrusts the body forward, leaving an attenuated, tail-like posterior end. This region is then pulled forward and the cycle of events is repeated. Apparently either end may precede in locomotion.

The Cercaria

The cercariae emerge from the sporocysts before they are entirely mature and complete their development in the lymph spaces of the snail. Most snails with old infections have cercariae free in the haemocoele. Normally emerged cercariae (Fig. 4) measure 0.2 to 0.5 mm. in length and 0.06 to 0.16 mm. in width. The acetabulum is 0.07 to 0.08 mm. in diameter. The acetabular sphincter is developed in the cercaria and the aperture of the sucker is transverse. The cuticula is spined. About the anterior end of the larva there are small papillae, each of which bears a fine bristle. The oral sucker is 0.06 to 0.065 mm. in diameter. The preoral region bears a simple, pointed stylet, 0.018 to 0.02 mm. long, which is directed anteriad. The mouth is subterminal; there is a short prepharynx and the pharynx measures 0.02 to 0.027 mm. in diameter. Its lumen is diagonal, from anterodorsal to posteroventral and in extended specimens the pharynx is usually longer than wide. The esophagus extends about half way to the acetabulum where it joins the digestive ceca which pass laterad and posteriad, ending blindly near the level of the anterior margin of the excretory bladder. In living specimens stained with neutral red the ceca are easily traced since they have a deep red color. The excretory system is fully developed in the cercarial stage. The location of the flame cells and ducts is shown on the right side of Figure 4. The bladder is lined with large epithelial cells and often contains refractive concretions. The reproductive organs are represented by two cellular masses (Fig. 4), one in front and the other behind the acetabulum. The anterior cells form the copulatory organs; the posterior cells give rise to the gonads, the female accessory structures and the uterus.

The unicellular glands of the cercaria are numerous and exceedingly hard to differentiate. The staining of living specimens with vital dyes and of fixed specimens with various cytoplasmic stains has not provided sufficiently clear distinctions for certain identification. The cells of any given type do not stain uniformly and in the penetration glands especially, the secretion may not stain at all in the cell body but stains more intensely as it passes along the duct. The age and condition of the cellular inclusions are apparently variable and the staining reaction varies accordingly. There are many cystogenous glands scattered over the body and they open to the surface both dorsally and ventrally. Near the posterior end of the body there are a number of glandular cells whose ducts pass posteriad and some of them open at or near the excretory pore. It is probable that these cells secrete the sticky material by which the larvae are attached. Anteriorly there are two clusters of glandular cells on each side of the body. There are about eight pairs of penetration glands, situated lateral and anterior to the acetabulum. Their ducts pass forward on each side of the body and behind the oral sucker may occasionally separate into three bundles, one of which passes mediad to the others which lie on the lateral side of the sucker. All open to the surface beside and below the stylet. The ducts are twisted about each other and their number could not be determined with certainty. Anterior and lateral to the penetration glands and partially overlapping them, there are other glands, probably six on each side, whose ducts pass forward and mediad, below the ducts of the penetration glands, and open into the prepharynx. The function of these glands is quite unknown.

The Metacercaria

Metacercariae were recovered from the parapodia and body wall of *Nereis virens* at intervals from one day to one month after the polychaetes were exposed to the cercariae. Each larva was enclosed in a thin, non-cellular capsule, produced by the cystogenous material of the cercaria. The body was bent ventrally, its dorsal surface applied to the cyst wall, with the two ends adjacent or overlapping. The parasites induced proliferation of fibroblasts in the tissues of the host and became enclosed in connective tissue capsules. When the host tissue was teased apart in sea water, the cysts fell out. The cysts were spherical to oval and immediately after encystment measured from 0.12 to 0.18 mm. in diameter. If the cysts were in locations where they were not subjected to pressure they remained spherical, if they were between muscle layers and compressed, they became oval. At the end of two weeks the cysts were noticeably larger, the larvae had grown, the excretory vesicles were filled with concretions, the gland cells were reduced but still recognizable, and the stylets were somewhat smaller. With the gradual resorption of larval structures, there was a corresponding development of the reproductive organs. These structures, represented in the cercaria by two groups of deeply staining cells, had begun to assume definitive form, although the gonads of metacercariae removed from Nereis four weeks after exposure to infection, were no further developed than those of the cercariae of *Z. lasius*.

Discussion

Life history studies require precise and accurate determination of the species of animals used in the investigation. In describing the worms from the tautog as a new species, *Zoögonoides laevis*, Linton compared them with the descriptions of *Z. viviparus* (Olsson, 1868) Odhner, 1902 as given by Olsson (1868), Odhner (1902) and Nicoll (1907). These accounts are at variance in certain respects. Odhner regarded Olsson's description as incorrect in certain particulars and Nicoll's description agrees in the main with that of Odhner. Although Odhner's measurements of the worm and of the oral sucker agree with those of Olsson, Odhner found the acetabulum twice as large as the oral sucker, an observation not in agreement with the findings of Nicoll. Olsson reported *Z. viviparus* as rare, he did not find it on the Swedish west coast and only two specimens were found in *Pleuronectes microcephalus* taken near Bergen, Norway. Both Odhner and Nicoll reported the parasites as abundant in several species of fish, chiefly flatfishes, and Odhner found it in *P. microcephalus.* Olsson reported the worms from the stomach, whereas members of the family Zoögonidae are typically parasites of the hindgut of fishes. Odhner regarded this observation of Olsson as "ein ganz zufälliges Vorkommens oder als ein Irrtum." It is not impossible that the specimens had developed in another host which had been eaten by *P. microcephalus* and that they had migrated from the hindgut of their host after it was eaten. Olsson's figure shows the opening of the acetabulum as oval, longer in the transverse direction; in Odhner's figure the aperture is nearly circular although the text states, "mit quergestellter, ovaler Lichtung." Describing the acetabulum, Nicoll found the "aperture nearly circular, or if elliptical the eccentricity is small." Olsson stated that the digestive ceca extend nearly to the excretory vesicle, whereas Odhner and Nicoll reported that the ceca do not extend past the middle of the acetabulum. In his figure, Olsson showed the genital pore as lateral, near the level of the intestinal bifurcation, but unfortunately there is no statement in the text and no legend to orient the figure; consequently it is impossible to determine positively whether the opening is on the right or left. Linton regarded Olsson's figure as a ventral view, with the genital pore on the right side. This interpretation is probably correct since in other of Olsson's figures, notably those of *D. fasciatum* and *D. increscens* in which the genital pore is lateral, statements in the text show that the figures were drawn from the ventral aspect. Furthermore, the protruding acetabulum of *Z. viviparus* would make it exceedingly difficult for a fixed and stained specimen to lie on the ventral side and consequently there are strong reasons for the opinion that Olsson's specimen was mounted with the ventral side up. Both Odhner and Nicoll, however, stated that in their specimens the genital pore is on the left side. Nicoll's measurements of the miracidium are larger than those of Odhner, whose figures agree with those of Olsson. In view of the disagreements in the descriptions, there seems to be a reasonable doubt whether Odhner and Nicoll had the same species that Olsson had described.

Linton accepted the accounts of Odhner and Nicoll as corrected descriptions of *Z. viviparus* and distinguished *Z. laevis* from *Z. viviparus* on the "comparative absence of spines" (a feature which he regarded as unimportant), the length of the digestive ceca, and the location of the genital pore. Where *Z. laevis* differs from the description of *Z. viviparus* as given by Odhner and Nicoll, it agrees with Olsson's original description of the species. The American specimens, described by Linton as *Z. laevis*, might reasonably be assigned to *Z. viviparus*, but such disposition would imply that the specimens of Odhner and Nicoll represent a different species, an inference that could not be justified without restudying their material. A more complete description of *Z. laevis* and knowledge of its life history will facilitate comparison with European species.

LITERATURE CITED

Linton, Edwin, 1940. Trematodes from fishes mainly from the Woods Hole region, Massachusetts. *Proc. U. S. Nat. Mus.*, **88**: 1–172.

Nicoll, William, 1907. A contribution towards a knowledge of the Entozoa of British marine fishes. Part I. *Ann. Mag. Nat. Hist.*, Ser. 7, **19**: 66–94.

Odhner, Th., 1902. Mitteilungen zur Kenntnis der Distomen. I. Ueber die Gattung Zoogonus Lss. *Zentr. Bakt., Parasit. u. Infekt.*, I, **31**: 58–69.

Olsson, P., 1868. Entozoa, iakttagna hos Skandinaviska hafsfiskar. *Lunds Univ. Års-skrift*, **4**: pt. 2, (8).

Stunkard, Horace W., 1938. Distomum lasium Leidy, 1891 (Syn. Cercariaeum lintoni Miller and Northup, 1926), the larval stage of Zoögonus rubellus (Olsson, 1868) (Syn. Z. mirus Looss, 1901). *Biol. Bull.*, **75**: 308–334.

Stunkard, Horace W., 1941. Specificity and host-relations in the trematode genus Zoögonus. *Biol. Bull.*, **81**: 205–214.

Stunkard, Horace W., 1942. The life cycle of Zoögonoides laevis Linton, 1940. *Jour. Parasit.*, **28** (Suppl.): 9–10.

INTERSEXUAL FEMALES AND INTERSEXUALITY IN HABROBRACON

P. W. WHITING

(From the University of Pennsylvania, Philadelphia, and the Marine Biological Laboratory, Woods Hole)

Much of the material discussed in this paper was collected by aid of a grant for assistance from the Penrose Fund of the American Philosophical Society. The female intersexes were found while work was being done under a grant from the Board of Graduate Education and Research of the University of Pennsylvania.

Despite the great amount of breeding work especially centering about a search for irregular sex types in the parasitic wasp *Habrobracon juglandis* (Ashmead), there has hitherto been reported (Whiting, Greb and Speicher, 1934) only one real intersexual form. This is the mutant type gynoid, the gene for which, gy, causes haploid males to be weakly intersexual. Gynoid females are indistinguishable from wild type. The trait acts as a recessive in heterozygous diploid males.

Gynoid males are similar to normal males in internal structure and in external genitalia. Their ocelli are large resembling those of normal males. Their normal male instincts indicate that the brain is structurally as in the male, since mating reactions in Habrobracon are determined by the brain. Sclerotization of the abdomen is progressively heavier anteriorly, approximating the condition found in the female. Antennae of normal males have about twenty segments in the flagellum, those of females usually not more than thirteen. In gynoid males the segments are reduced in number to that of the female, although they are not quite as short and thick. Superficially a gynoid male suggests a sex-mosaic or gyander with female head, male abdomen, but, as indicated, certain structures are themselves intergrading, the body is approximately symmetrical with all parts presumably of the same genetic constitution and the type is perpetuated as a pure-breeding form.

Nine intersexual females of the same species have recently been found and are herewith reported for the first time. They occurred among the offspring of a single female. Superficially these appear to be the reverse of the gynoid males, being more masculine anteriorly, feminine posteriorly. The heads are characteristically male having large ocelli and long antennae, flagellar segments ranging from 18 to 21 with 20 as the mode. Tests made on five of the nine showed indifference to caterpillars and vigorous attempts to mate with females, indicating the brain to be structurally male. Abdominal sclerotization is male-like anteriorly. The first and second tergites are thin and the anterior sternal thickenings small. Sclerotization is progressively heavier posteriorly and sternal thickenings become elongate, approximating the condition of the female.

Internal structures of the abdomen are as in the female, including normal poison sack and glands and seminal receptacle. The ovaries, however, lack differentiated nurse cells and ova. Each appears to be a pair of sacks of oögonia similar to the primordia of the ovarioles formed in the spun-in larva and normally remaining essentially unchanged until the eyes of the pupa begin to turn black, when differentiation of oöcytes begins.

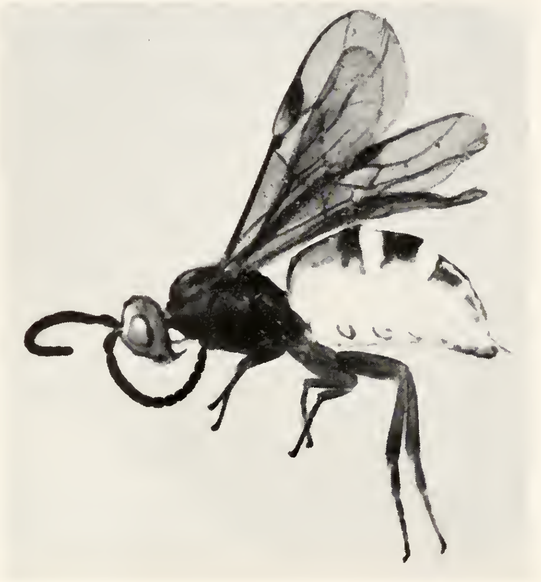


FIGURE 1. Gynoid male. Note the short "female" antennae and the heavy anterior sclerotization of the abdomen. ×16.

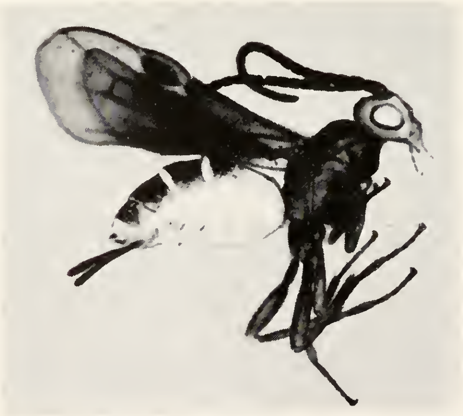


FIGURE 2. Intersexual female. Note the long "male" antennae and the decrease in abdominal sclerotization anteriorly. ×16.

Like gynoid, these intersexes differ from sex mosaics in being approximately symmetrical and similar to each other, in possessing sex intermediate characters and in occurring in a group in one fraternity as if caused by an hereditary factor rather than being scattered as single individuals.

The fraternity containing these nine intersexes was small since the mother had been discarded after eight egg-laying days,—two vials. Offspring were being classified for sex and for certain eye colors. Nothing unusual was observed in the first vial from which were obtained 13 females (+ 10, orange 3) and 11 males (+ 3, orange 8) of expected types. The nine intersexes (+ 7, orange 2) were all found in the second vial which contained 13 males (+ 4, orange 9) and no females. Proportion of wild type to orange eye color deviates from the expected 1 : 1 in the females and intersexes in the opposite direction from that in the males. This may be but a fluctuation due to small numbers or it may indicate some chromosomal irregularity.

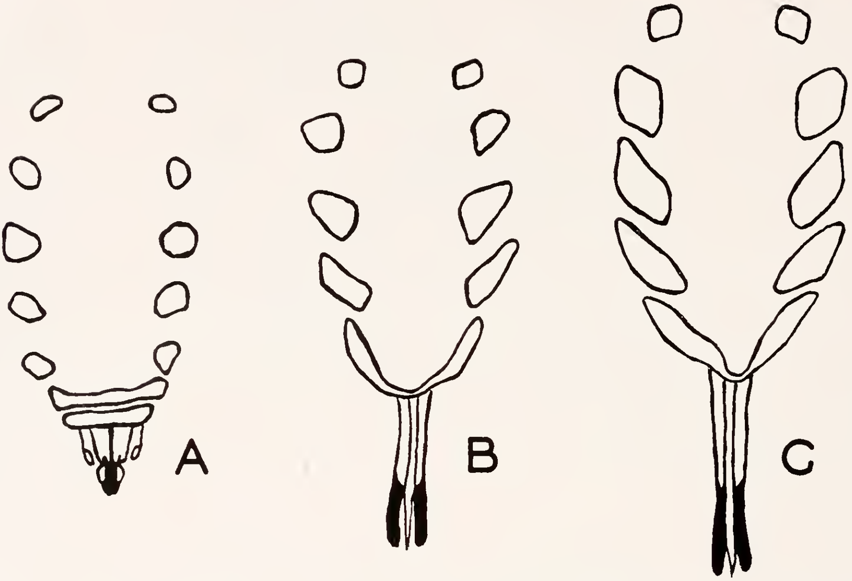


FIGURE 3. Outlines of abdominal sternal thickenings and of external genitalia in normal male (A), intersexual female (B) and normal female (C). ×65.

The males appeared structurally normal except that one had external genitalia slightly reduced, a condition not infrequently found in Habrobracon. Dissection of seven including this one showed internal genitalia normal. Flagellar segments of antennae ranged from 18 to 21 with mode 20, normal for males. Ocelli were of normal male size.

Since the offspring in the first vial had been discarded no tests could be made. A mass culture from the vial 2 males, besides individual pairings of four of these with related females, yielded nothing irregular in the immediate progeny or in later generations. Many closely related side lines, which were being bred for the eye color studies, gave only normal types. Unfortunately the eye colors were brought into the mutant fraternity in such a way as to be of no significance for determining which offspring were from fertilized, which from unfertilized eggs.

DISCUSSION

The mother of these intersexes may have mated with two different brothers before she was isolated for breeding. Sperm from one male may have been used first to produce the females. The second male may have sired the intersexes from sperm with a dominant intersex factor. This hypothesis is regarded as unlikely in view of the fact that the females and intersexes were produced in separate vials. In known instances of double matings the two types of offspring expected eclose together, suggesting that the sperm have mixed.

If a mutation occurs in a primitive germ cell of a female of Habrobracon, the resulting mutant tissue tends to form a stratum cutting across the two ovarioles of both ovaries. This is due to the method of development from the primitive germ cell mass which separates longitudinally into the gonad primordia in a late embryonic stage. Each primordium elongates in the grown maggot, the beginning of sex-differentiation, and is subsequently divided longitudinally into two sacks, which become the ovarioles. Non-mutant tissue may then function for a period producing eggs in the first vial, for example, while heterozygous mutant tissue gives rise to eggs produced later.

If the intersexes be regarded as haploid (male) from unfertilized eggs, it may be supposed that the mother's sperm supply was exhausted before transfer to a second vial. Normal males and intersexual males would then segregate in equal ratio in vial 2. This hypothesis is regarded as unlikely because of the structure of the intersexes indicating that they are fundamentally female.

A dominant mutation in the ovaries should appear in only half of the zygotes produced while the mutant tissue is functioning. Normal females might then be expected in vial 2 at least equal in number to the intersexual. If, however, the mutation occurred in the sex-differentiating chromosome segment as a modification (deletion ?) in one of the sex factors (changing xb to xb^m), females (xa/xb) might be replaced by intersexes (xa/xb^m). Sperm, xa, fertilizing eggs from xa/xb tissue in vial 1 would produce normal females, xa/xb, and diploid males, xa/xa, but from xa/xb^m tissue in vial 2, intersexual females, xa/xb^m, and highly inviable diploid males, xa/xa, would result. Unfertilized eggs would give haploid males as expected but xb^m males might be inviable.

The data must be regarded as inadequate to prove whether these intersexes were due to a modification connected with the normally sex-differentiating factor or whether, like gynoid, to an independent change. The series of sex alleles, xa, xb, xc, etc., has been shown to be located at about the center of the left arm of the linkage map. The gene gynoid, gy, is located near the distal end of the right arm and therefore segregates independently of sex.

It is questionable whether the diverse effects of gynoid on antennae and abdominal sclerotization should be regarded as multiple effects of a single gene. Gynoid may possibly be a translocation from the differential segment determining sex, the x factor. In a male with the sex allele in the normal position this might give a complementary feminizing effect causing intersexuality.

Goldschmidt has defined an intersex as a phenotypic mosaic which begins development as one sex according to its chromosomal constitution, XY or ZZ ♂, XX or WZ ♀, and then, after a turning-point, forms organs as in the opposite sex. The earlier the turning-point, the higher the degree of intersexuality.

With sex determination as in Habrobracon, haploid intersexes should begin development as male, later shifting to female. The same should apply to diploids if homozygous for the sex factor. Diploids heterozygous for sex should begin development as female, later shifting to male.

The nine intersexual females discussed here must be regarded as more strongly intersexual than gynoid males since antennae, ocelli and instincts are completely sex reversed. The abdominal sclerotization of both intersexual types is intermediate. Neither external nor internal genitalia are affected except that the ovaries of the intersexual females fail to mature, remaining as sacks of oögonial tissue. The turning-point appears to occur earlier in the anterior than in the posterior region of the body or else the developmental processes forming the external genitalia, which are begun before the turning-point, are such that they must be carried to normal completion.

A comparison may here be made with triploid females of Habrobracon. These are daughters of diploid males arising from fertilization of a normal egg by a diploid sperm. They are presumably an unbalanced type, having two similar paternal sex alleles combined with a dissimilar member of the series of maternal origin. A diploid female may then be xa/xb, while a triploid will be xa/xa/xb or xa/xb/xb. These formulae suggest the possibility of intersexuality, but dominance relationships appear to be such that triploid females show no masculine traits either in structure or in reactions.

Their egg production is, however, considerably lowered, being about one-fourth that of diploid females. This reduction, involving both ova and nurse cells, may be a small step toward intersexuality. A compensatory growth takes place in the oögonial chamber which enlarges and elongates considerably under the influence of feeding from host caterpillars. It would be of interest to know what might have happened to the ovaries of the intersexual females if they had been similarly nourished.

The suggestion has frequently been made that diploid males may be sex-reversed females. This view originates in the older concept that diploidism as such causes femaleness, a view now shown to be erroneous. Certain differences of diploid from haploid males are to be expected dependent upon chromosome number, but these are not necessarily in the direction of femaleness. Cell-size for example is not only much greater in diploid males than in haploid: it actually surpasses considerably the cell size of the normal diploid females. The sex-linked gene "fused" causes antennal segments to be much reduced and fused together. Fused females have much shorter antennae than haploid fused males. Diploid fused males approximate fused females, having antennae only slightly longer. Wild type females have much shorter antennae than haploid wild type males. Diploid wild type males approximate haploid, but, as determined by count of segments, their antennae are slightly shorter. Difference in antennal length of diploid from haploid males is due to chromosome number as such; difference of females from diploid males is due to heterozygosis for the sex factor. As regards antennae, the gene "fused" increases the difference due to chromosome number, but tends to mask the difference due to sex. Intermediacy of diploid males with respect to antennal length should not be regarded as intersexuality.

SUMMARY

Nine female intersexes are described and compared with the one intersexual form previously known in Habrobracon, the fertile mutant type gynoid, a weakly intersexual male.

These female intersexes proved sterile, having male heads and instincts and abortive ovaries. They are, in general, female posteriorly, while gynoid males have partially feminized heads but react like males.

Evidence suggests a dominant mutation in the sex-differentiating factor as a possible cause of this female intersexuality.

On the basis of comparison with these female intersexes, it is suggested that the structure of ovaries in triploid females represents a step toward intersexuality.

Antennal length of diploid males, both fused and wild type, although intergrading, is not regarded as due to intersexuality but to diploidy as such.

LITERATURE CITED

WHITING, P. W., RAYMOND J. GREB AND B. R. SPEICHER, 1934. A new type of sex-intergrade. *Biol. Bull.*, **66**: 152–165.

NEW RECORDS OF SOME MARINE CHAETOPHORACEAE AND CHAETOSPHAERIDIACEAE FOR NORTH AMERICA *

FRANCESCA THIVY

(*University of Michigan, Ann Arbor, Michigan*)

The study of the microscopic epiphytic, endophytic, shell-boring, and lithophilic Chlorophyceae—a habit group not as yet fully explored for Woods Hole—was suggested by Dr. Wm. Randolph Taylor and was carried out, under his direction, during the summers of 1939 through 1942, at the Marine Biological Laboratory. For helpful criticism the writer is greatly indebted to him.

In making this second report of the investigation, the writer wishes to express her sincere gratitude to the Levi Barbour Foundation of the University of Michigan, and Dr. Alma G. Stokey, through whose kindness these studies were made possible.

Of the five members which are here added to the marine Chaetophoraceae of North America, four have been described for Europe and one for the West Indies. The present report of *Diplochaete solitaria* Collins (Chaetosphaeridiaceae) for Woods Hole, Massachusetts, extends its distribution north of Jamaica.

CHAETOPHOREAE

Phaeophila Hauck, 1876. Plants endophytic, immersed within the external cell walls or embedded in the cortex of the host, shell-boring, or rarely epiphytic; thallus forming discs consisting of free or partly fused uniseriate branches; branching lateral, alternate; cells cylindrical to round, often sinuous or with irregular protrusions, frequently setigerous, with usually several nuclei; chloroplast parietal, plate-like, partly lining the cell wall, lobed at the margins, at times becoming perforate or breaking up into discs; pyrenoids 1–13; setae usually one to a cell, occasionally two or three arising from a cell, firm-walled, distinct, very long, usually wavy but often straight while within the host and wavy outside it, without a basal septum but often developing a thick collar at the base and at times thereby becoming occluded, or, seta secondarily developing a basal septum; sporangia intercalary, or terminal on branchlets, cylindrical, round or conical, containing many zoöspores, provided with a wide cylindrical neck; sporangial neck twice the diameter of a seta but shorter, without wavy walls; zoöspores escaping together or one after the other, quadriflagellate; biflagellate zoöids observed in only one case (Huber 1892b, pp. 330–31); entrance into the host effected by a germination tube arising from the anterior end of the zoöspore; zoöspore and the lower end of the germination tube becoming empty and later cut off by a septum.

---

* Paper from the Department of Botany, University of Michigan, No. 733.

Phaeophila Engeri Reinke
Phaeophila divaricata Huber, 1892b, p. 331.
Ochlochaete Engleri (Reinke) Hansgirg, 1892, p. 201.

Plant inhabiting living or dead shells of marine annelids and molluscs, imparting a grass green color to them, present on both sides or only on the dorsal side of the shells often associated with other algae; thallus visible only after decalcification, consisting of branched procumbent filaments extending in various directions; cells occasionally showing one to several rhizoidal processes about 4.8 μ diameter (Plate II, figure 14); central cells deeply lobed, partly fused and forming a network, but the filaments distinct peripherally; branching lateral; cells cylindrical and either straight or sinuous along their length, or isodiametric with very irregular lobes; cells 4.7–21.6 μ diameter, 1–5 times as long, when isodiametric and irregular up to 52.4 μ wide; in Spirorbis shells, however, cells only 4.7–11.8 μ diameter, 1–5 times as long, when isodiametric and irregular up to 13.2 μ wide; setae not numerous, sometimes lacking, either sinuous or straight, continuous with the lumen of the supporting cell or secondarily separated by a cell wall from it, both when basally open and when closed showing sparse granular contents; setae when open at the base, often strengthened by a collar-like basal thickening of the wall; width of setae 1.18–2.55 μ, sometimes 3.6 μ; length of setae about 0.1 mm; cells 1–5 nucleate, with 1–7 pyrenoids; chloroplast parietal, plate-like with lobed margins, at times breaking up into discoid portions, often crowded with starch grains; cells frequently sending out 1–5 globular to oval, vertical or lateral processes capable of developing an apical cap-like, stratified swelling of the cell wall (Plate I, figures 1 and 2); or cells, in some instances, bearing directly a similar or a peg-like thickening of the wall associated with a seta or alone (Plate I, figures 9, 11, 15 and 16); end walls of cells occasionally stratified; lateral walls sometimes having a number of small lentiform swellings (Plate II, figures 7–10); sporangia flask-shaped to irregular, usually intercalary, 10.2–28.05 μ long, 1–1½ times high, provided with a cylindrical neck about twice the diameter of a seta, 3.53–5.32 μ wide and the length 1–7 times the width; zoöspores 6–22 in number, ovoid, sometimes spindle-shaped, when swimming measuring 3.6–8.4 μ in diameter, 1½–2 times as long, quadriflagellate, having a pyrenoid, an eye-spot and a pair of contractile vacuoles; length of the flagella usually equalling that of the zoöspore; germination occurring with the formation of a tube from the anterior end of the zoöspore in a line with its long axis; the zoöspore and the lower part of the germination tube becoming empty and cut off by a septum; aplanospores rarely seen, about 16 in a cell, each surrounded by a cell wall, 7.11–9.41 μ diameter.

Woods Hole, Massachusetts: On clam shells (*Mya arenaria* L.)—Black Rock, 23 July 1941, coll. W. R. Taylor, July 1942; Great Harbor, 15 July 1941 and 14 Aug. 1942; Penzance, salt marsh, coll. Jennie L. S. Simpson, 26 Aug. 1942; on quahaug shells (*Venus mercenaria* L.), Black Rock, 23 July 1941; on *Busycon carica* Gmelin, Great Harbor, coll. W. J. Gilbert, 30 July 1941; on *Polynices duplicata* (Say), Spindle, 26 Aug. 1942; on *Anomia simplex* D'Orbigny, Spindle, 26 Aug. 1942; on *Thais lapillus* (L.), Gay Head, 16 July 1941; on *Spirorbis spirorbis* (L.), attached to *Fucus vesiculosis* L., Spindle, 26 Sept. 1942; all excepting Spirorbis and Thais were dead shells.

Europe: On *Spirorbis nautiloides*—Kieler Föhrde, Engler and Reinke; Bülk, Baltic Sea, Lakowitz; on Spirorbis and shells of various mussels and snails, Kristineberg, Swedish west coast, Kylin; Weymouth, Dorset, Engl., Batters.

Distribution: Baltic Sea, southern coast of England, Atlantic coast of N. America.

Reinke, 1889, p. 86; Batters, 1902, p. 13; Migula, 1907, p. 807; Lakowitz, 1929, p. 138, figure 194; Kylin, 1935, pp. 193–97, figures 3, A–F and 4, A–M.

P. Engleri is very similar to *P. dendroides* (Crouan) Batters, but is readily recognized by its shell-inhabiting nature, the latter species being endophytic in various marine algae. Reinke considered (1899, p. 86) that the sinuous cells of *P. Engleri* distinguished it from *P. dendroides*. Though the latter also often has deeply sinuated walls, a greater variability of cell shape and wall is characteristic of *P. Engleri*. A cell of *P. Engleri* may bear several lateral and vertical papilla-like processes which give it an extremely irregular contour (Plate I, figures 1 and 2). As described by Kylin the processes may develop an apical thickening of the wall, which breaks through the shell layers and communicates with the external medium. When the papillate processes are lacking, evidence of the tendency to form connections with the exterior is seen in the cap-like or conical pegs formed directly on the cells as described above. *P. dendroides* was found growing in the walls of *Chondrus crispus* (L.) Stackh., *Polysiphonia flexicaulis* (Harv.) Collins, and *Champia parvula* (C. Ag.) Harv., at Woods Hole, Mass. It does not have papillate cell processes, but its cells often have a solitary

---

PLATE I

Phaeophila Engleri Reinke

From Spirorbis

FIGURE 1. Filaments showing three papillate cell processes with apical thickening of the wall, × 1000.

FIGURE 2. A cell with 5 processes resembling cells in Bor. et Flah., 1889, Plate VI, Figure 3; other cells showing chloroplast, pyrenoids and starch grains, × 1000.

From Urosalpinx

FIGURE 3. Filaments in natural position, × 481.

FIGURE 4. Filament with a developing sporangium, × 481.

FIGURE 5. Cell filled with starch grains, × 681.

FIGURE 6. A terminal sporangium, × 481.

From Mya

FIGURE 7, a–e. Zoöspores; *a*, living, showing chloroplast and eye-spot; *b–e*, stained with iodine; *d*, unusually large zoöspore with 2 contractile vacuoles, eye-spot and pyrenoids, × 929.

FIGURE 8. Intercalary sporangium with aplanospores, × 710.

FIGURE 9. Filament showing a sporangium with a peg on the wall, by the side of its neck, × 481.

FIGURE 10. Filament showing a sporangium with a seta beside the neck, × 471.

FIGURE 11. Sporangium with a peg and a narrow neck, × 1000.

FIGURE 12. A globular sporangium, × 481.

FIGURE 13. Empty sporangia, × 763.

FIGURE 14. Two irregular cells embedded deeply in the shell, × 734.

FIGURE 15. Cell with a seta and a dome-shaped tubercle on the wall, × 1000.

From Polynices

FIGURE 16. Cell with a seta and a tubercle on the wall, × 1000.

FIGURE 17. Sporangium with zoöspores showing eye-spots and pyrenoids, × 547.

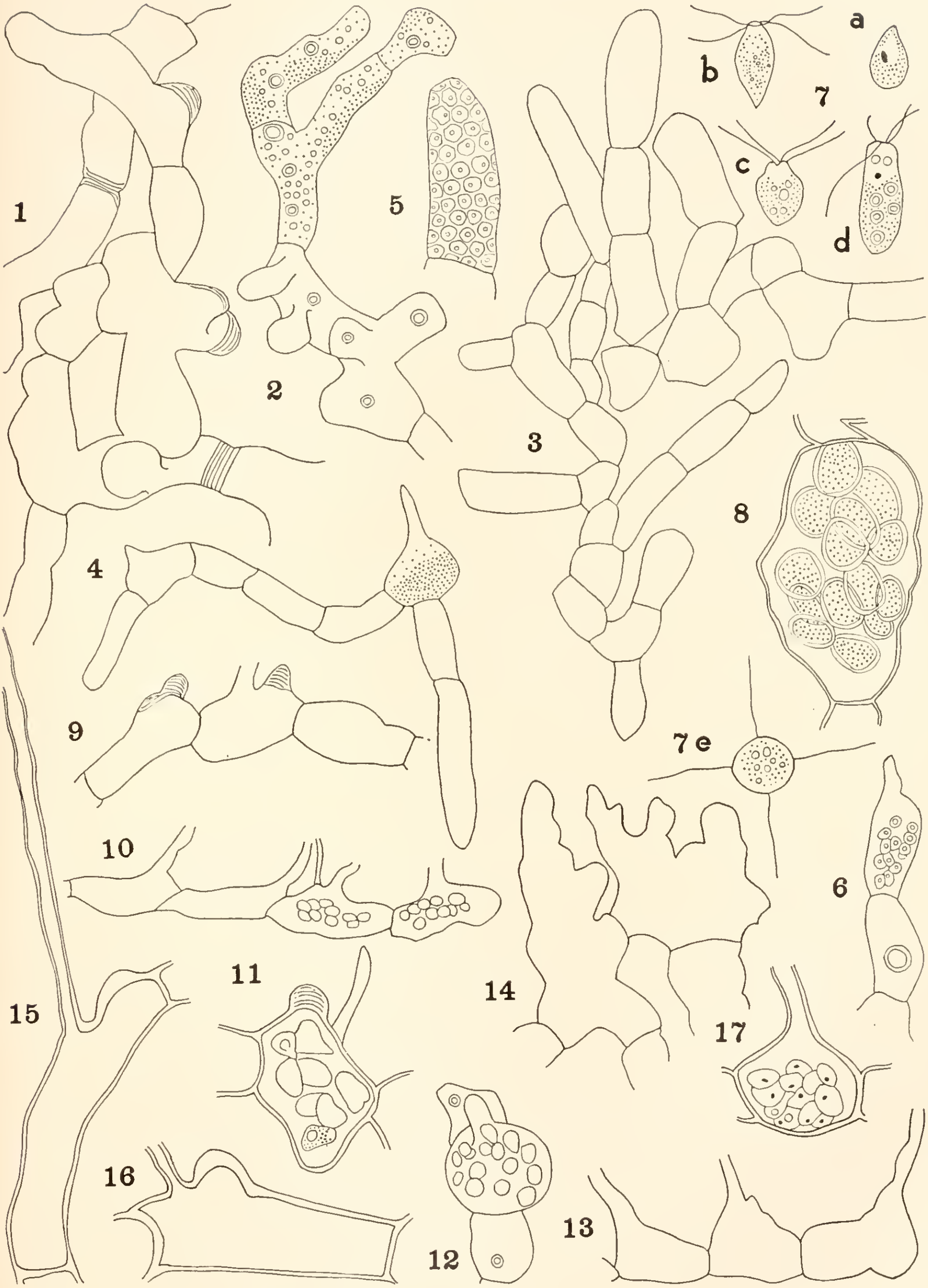

a
b
7
c
d
1
5
2
3
8
4
9
7 e
6
10
15
11
14
17
16
13
12

PLATE I

lateral process resembling the initial stage of a branch; the peculiar swellings of the wall found in *P. Engleri* are completely absent.

Another difference between the two species is discernible in their sporangia. In both they are usually intercalary, cylindrical to flask-shaped, but the length of the emission tube is much shorter in *P. Engleri* (Kylin, figure 4L) than in *P. dendroides* (Huber, Plate XVI, figure 9). It is either a short papilla or a tube in length about 1–2 times the height of the cell in the first case, and about 4 times the height of the cell in the other. In *P. dendroides* it extends beyond the host to about 25.5 μ as seen in the examples at Woods Hole, but in *P. Engleri* the tube apparently does not project beyond the shell surface. It is significant that figure 4L cited above represents sporangia in a culture, *without the shell*, as it affords evidence that the short emission tube is a stable character of the species.

The phenomenon of setae secondarily developing a basal wall found in *P. Engleri* may also be seen, though very rarely, in *P. dendroides*.

That chalk-boring algae are of greater importance than animals of similar habitat in breaking down calcareous substrata and releasing potassium, magnesium and other elements, is stated by Nadson (1927, p. 153). He says that various blue green algae as well as *Gomontia polyrhiza* (Lagerh.) Born. et Flah. and *Ostreobium Queketti* are widely distributed, but not *P. Engleri* and *Conchocelis rosea* Batters. One may conclude from the common occurrence of *P. Engleri* at Woods Hole and presumably also in Europe, the alga probably is present in many more localities than are so far known. In all cases of clam and quahaug shells examined for *P. Engleri*, at Woods Hole, the latter alga was mixed with the large unicells of Gomontia, but in Anomia, Polynices, Thais and Spirorbis, *P. Engleri* was present alone.

Plate II

Phaeophila Engleri Reinke

From Mya

Figures 1–2. Cells showing setae with the secondary basal septum; in Figure 2 cell contents seen in the seta; Figure 1 × 1000; Figure 2 × 592.

Figure 3. Cell showing seta with a collar-like basal thickening of the wall and cytoplasmic granules, × 1000.

Figures 4–5. Cells showing basally open setae, Figure 4 × 493; Figure 5 × 592.

Figure 6. Cell with two setae, one basally open, the other with a basal septum, × 592.

Figures 7–10. Cells showing lenticular swellings in their lateral walls, Figure 7 × 751; Figure 8 × 586; Figure 9 × 624; Figure 10 × 724, from a culture on shells.

Figure 11. Filaments showing narrow cylindrical, and large globular cells, × 666.

Figure 12. Filament showing irregular cells resembling in shape and size some of the cells in Bornet and Flahault, 1899, Plate VII, Figure 16; × 1142, from a culture on shells.

Figure 13. From a culture on shells, an intercalary cell showing a large cylindrical process resembling in size the spindle-shaped cell process in Taylor, 1937, Plate I, Figure 13, and agreeing in size as well as shape with cell processes in Bornet and Flahault, *l. c.*, Plate VII, Figures 14 and 16; × 703.

Figure 14. Cells with rhizoids as in *l. c.*, Plate VI, Figure 7; × 634.

From Anomia

Figure 15. Filament showing several nuclei to a cell, as in *l. c.*, Plate VIII, Figure 20; × 813, from a culture on shells.

From Urosalpinx

Figure 16. Filaments with two flask-shaped young sporangia, × 813.

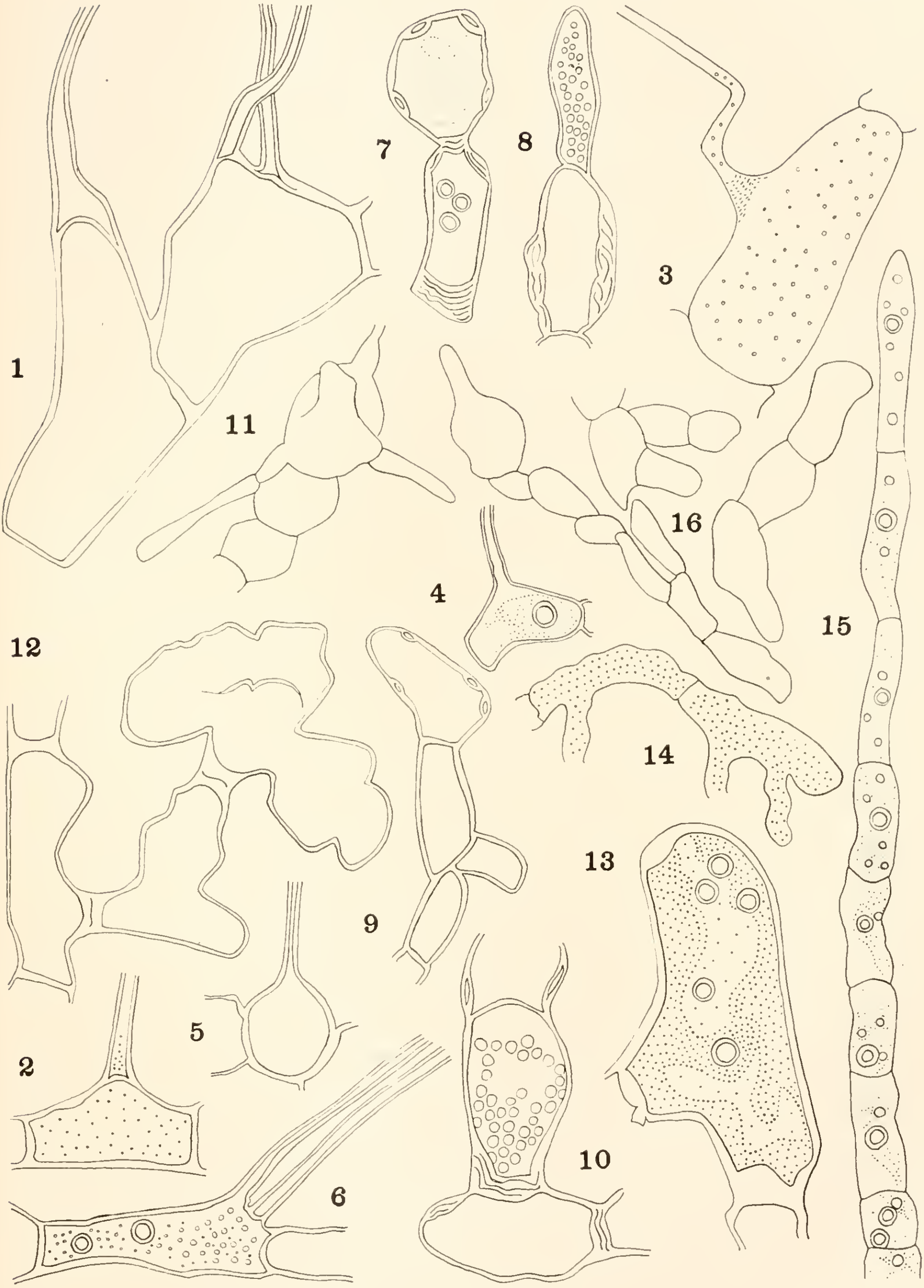

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

PLATE II

Because of the frequent occurrence together of *Gomontia polyrhiza* and *Phaeophila Engleri*, the swollen cells of the latter could be taken to represent the intermediate stage in the development of intercalary cells into the unicells of Gomontia, for which such an origin has been described by Bornet and Flahault (1888, pp. 163–65; 1889, pp. CLII–CLX, Plates VI–VIII). Observations of *P. Engleri* and *G. polyrhiza* made in the course of this study and a comparison of these algae (cf. Plate II, figures 12–15) with the descriptions and figures of *G. polyrhiza* given by Bornet and Flahault (op. cit.) for the Atlantic coast of France and the Mediterranean Sea and by Taylor (1937, pp. 57–58, Plate I, figure 13) for the Atlantic coast of North America, suggest that the filaments attributed to Gomontia are, with little doubt, the filaments of *Phaeophila Engleri*. The latter alga has probably often been overlooked and its distribution must be at least as wide as that of *Gomontia polyrhiza*.

P. divaricata Huber (p. 331, Plate XVI, figures 12 and 13) agrees with *P. Engleri* in cell size and in its calciphilous habit within the encrusted walls of the 'stems' of Acetabularia. The sporangia of the two species are very similar, and characteristically different from those of *P. dendroides;* the form on Acetabularia is probably the same as *P. Engleri Reinke*. It is of interest also to note, since *P. Engleri* has evidently been taken to be a stage of Gomontia, that Huber says some of the cells of *P. divaricata* are swollen toward their summits and resemble the habit of Gomontia.

P. dendroides var. *calcicola* Hansgirg growing in the shells of gastropods and also in Corallina and Lithothamnium is reported for Istria and Dalmatia (Hansgirg, 1892, p. 201). Taking into account Kylin's remark (p. 194) that *P. Engleri* is more common at Kristineberg on the shells of various mussels and snails than on Spirorbis, it appears likely that *P. dendroides* var. *calcicola* is synonymous with *P. Engleri*.

The present observations show that the forms of Phaeophila, occurring on Spirorbis on the one hand, and in the shells of molluscs on the other, cannot be considered as different species as they vary only in cell size. The smaller dimensions of the perforating form on Spirorbis may result from space limitation.

Ectochaete (Huber) Wille, 1909. Species marine or fresh-water; plants microscopic, endophytic in other algae; thallus usually forming discs of radiating partly fused filaments within the host wall, in one species developing pseudoparenchymatous cushions after emerging from the host wall, in another anchored in the mucilage investing the assimilatory filaments of the host in the form of threads interwoven with those of the host; branching lateral or rarely dichotomous; cells uninucleate, cylindrical to round, setigerous; seta solitary, straight, very long, showing sparse granular contents, without a basal septum but at times the lumen not visible at its base; chloroplast single, parietal, incompletely cylindrical, plate-like or sometimes perforate, usually with numerous starch grains; pyrenoids 1–8; plants reproducing by zoöspores or isogamous gametes; zoösporangia or gametangia developing from vegetative cells, having a short to long beak and containing many zoöids; zoöspores and gametes biflagellate, with a chloroplast, a pyrenoid and usually an eye-spot; germination either accompanied by the production of a germination tube from the anterior end of the zoöspore or not.

Ectochaete leptochaete (Huber) Wille. Plant filamentous, endophytic in the external cell walls of green, brown and red algae, forming minute monostromatic discs 64–166 μ diameter; branches lateral, irregularly alternate, arising as a rule near the proximal end of a cell, often with the basal septum occurring a short distance above the subtending cell, in the older stages showing fusions in the center of the disc but filaments remaining free outwardly; central cells with a diameter of 5.88–11.82 μ (according to Huber, 5–15 μ), isodiametric or 1.5 times as long; cells towards the ends of the filaments usually 3.5–4.7 μ diameter, 2–4 times as long; setae very delicate, tubular, containing cytoplasmic granules, 1.2–1.5 μ diameter, about 80 μ in length, with a constriction at the point of exit from the host wall; lumen of seta continuous with that of the supporting cell but sometimes not visible at its base, the wall being opaque at this point; chloroplast plate-like, incompletely lining the wall, perforate in the older stages; pyrenoids usually 1–3, sometimes 4; many central cells serving as zoösporangia, 5.88–11.82 μ diameter, round, conical or shortly cylindrical with a short, vertical, hyaline beak, in length usually one third the diameter of the cell and rarely half the diameter; zoöspores about 12 in each, 2.4 μ diameter, either spherical or ovoid, 4–5 μ long, biflagellate, with flagella three times the cell length; during germination the anterior end of the zoöspore prolonging into a germination tube and entering the host, the empty zoöspore not cut off by a wall (Huber 1892b, Plate XV, figures 8 and 9).

Woods Hole, Massachusetts: In the external walls of *Polysiphonia novae-angliae* Taylor, especially in the basal part of the host, in the wash at Nobska Point, September 1 and September 10, 1942; in *Chondrus crispus* (L.) Stackh. (intertidal) and the leaves of *Sargassum Filipendula* C. Ag., from the wash at Nobska Point, 10 September 1942.

Huber recorded the species for the Gulf of Lyons where it was present in salt water ponds, on Cladophora, *Chaetomorpha Linum* and *Ceramium diaphanum* (Lightf.) Roth, (Nov. and April), and for the Bay of Biscay, on a Chaetomorpha (Sept.). It is cited by Feldmann for the Gulf of Lyons, as growing in *Dictyota dichotoma* and *Dilophus Fasciola*, (May to June). For the English Channel, we have reports by Hariot, on *Cladophora tenerrima* at Tatihou, by Batters from Devon, and by Newton, on *Ectocarpus penicilliformis*, *Ceramium diaphanum* and Cladophora, also from Devon.

Distribution: W. Mediterranean, Atlantic Coasts of France, England and N. America.

Huber, 1892b, pp. 319–26, Plate XV, figures 1–9; Batters, 1902, p. 14; Wille, 1909, p. 79; Oltmanns, 1922, I, pp. 299–300; Printz, 1927, p. 194; Hamel, 1930, p. 28; Newton, 1931, p. 62; Feldmann, 1937, p. 181; Thivy, 1942, pp. 98 *et seq.*

The species may be distinguished from *E. Taylori*, another marine species to which it comes nearest in structure, by its habit and by the delicate setae only half as wide as those of *E. Taylori*, which has setae 2.66–3.8 μ in diameter, as well as by its smaller cell size, for in *E. Taylori* the cells have a diameter of 8–18 μ and sometimes up to 25.5 μ. Besides, each of the two species is characterized by its sporangium and by its method of germination. The careful and detailed description given by Huber 1892b applies closely to the alga as found at Woods Hole, except that the cell diameter does not reach 15 μ in the present case.

In cultures without the host, grown in sterile 0.875 Detmer's solution, the species behaves, on the whole, like *E. Taylori*, forming a disc from which upright, branched filaments arise in the place of the setae and grow into a fuzzy mass about 1 mm. in diameter. The vegetative cells and sporangia are larger than in nature and reach 23.52 μ diameter. The zoöspores are also larger, 3.5–5.1 μ in diameter, and there are up to 30 in a sporangium. When the culture is transferred to plain sterile sea water, setae appear after the lapse of a week. The basal constriction observed by Huber both in nature and in cultures of the endophyte still within the host, is seen in the above cultures, though the host is absent.

Ectochaete vagans (Börgesen) *comb. nov.* (*Endoderma vagans* Börgesen). Thallus filamentous, microscopic, endophytic in the external cell walls of various algae, creeping; filaments uniseriate, forming a network or radiating from a center, fused to a slight extent in the middle of the thallus or free throughout, widely spreading at their ends; branches lateral, irregularly alternate, sometimes without a basal septum; cells sub-cylindrical, often having at the middle or at the upper end a lateral obtuse outgrowth remaining as such or growing into a branch, 5.32–13.3 μ diameter, 1–4 times as long, with the diameter at an outgrowth usually doubled; end cells of attenuate filaments 1.23–5.32 μ and 5–17 times as long; occasionally intermediate cells may be narrower and longer than normal; plants having on their walls, either laterally or vertically, dome-shaped, conical or peg-like tubercles in width a third to half the diameter of the cells, but lacking them sometimes; an extremely fine, long, straight seta, 1.176 μ in diameter, about 100 μ long, proceeding from the conical tubercle; seta continuous with the lumen of the supporting cell, tubular but the lumen usually not visible and when in view seen only above the opaque base of the seta, deciduous; conical base of seta about 8 μ diameter, embedded in the host wall, incrassate, usually transversely striate or punctate with less refractive areas; chloroplast parietal,

---

PLATE III

Ectochaete leptochaete (Huber) Wille

From *Polysiphonia novae-angliae* Taylor

FIGURES 1–2. Habit of two plants, Figure 1 × 749; Figure 2 × 855.

FIGURE 3. Sporangia (necks not visible) and uninucleate cells in nature, in surface view, × 855.

FIGURE 4. Habit of plant in culture (0.875 Detmer solution) showing branches in the place of setae, × 24.

FIGURE 5. Filament from a culture (0.875 Detmer solution) bearing a long two-celled erect branch in the place of a seta, × 394.

FIGURE 6. Terminal colorless cell ending in a basally open seta (sea water culture), × 855.

FIGURE 7. Terminal green cell ending in a basally open seta (sea water culture), × 855.

FIGURE 8. Intercalary cells with setae showing continuity of the lumen from cell into seta (sea water culture), × 855.

FIGURE 9. Empty sporangia with rather long necks (culture in 0.875 Detmer solution + algal extract), × 394.

FIGURES 10–11. Sporangia with necks of average length filled with a semi-hyaline wall substance. (Culture in 0.875 Detmer solution + algal extract), Figure 10 × 444, Figure 11 × 394.

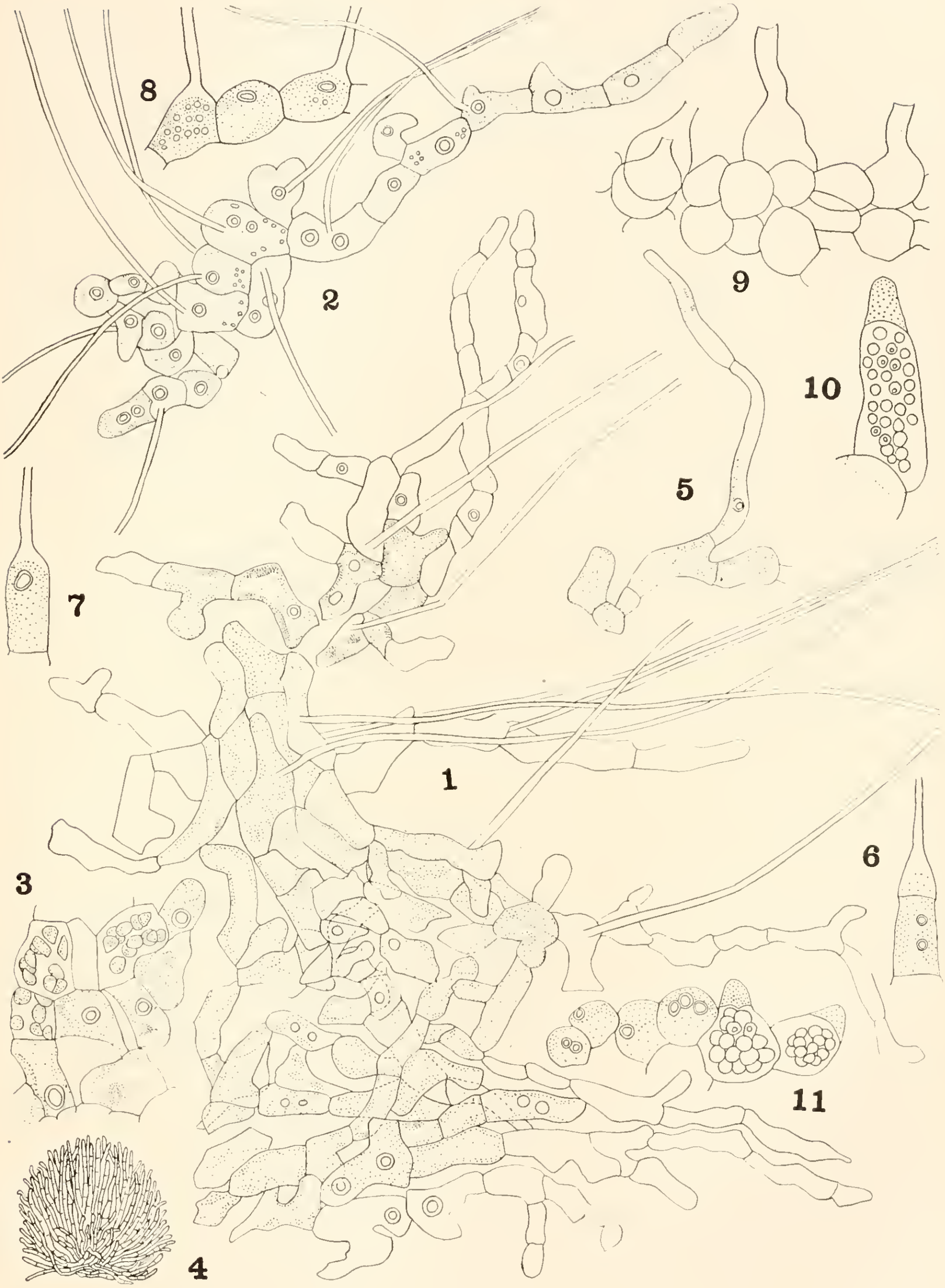

8
2
9
10
5
7
1
6
3
11
4

PLATE III

partly lining the cell wall, in older cells obscured by the numerous starch grains, appearing bright green in dry specimens; pyrenoids 1–5, usually 1–3 (Börgesen found 5–7); sporangia arising from usually somewhat enlarged vegetative cells, producing 7–15 zoöspores; the latter 2–3 μ diameter, 4–4.7 μ long; in the sporangia provided with tubercles the opening occurring through them; the number of flagella not observed so far; sexual reproduction not known; during germination the anterior end of the zoöspore growing in a line with its long axis and forming a germination tube, the latter becoming the first green cell.

Woods Hole, Massachusetts: In the wall of *Polysiphonia flexicaulis* (Harvey) Collins, coll. Gladys Bulmer, in the wash at Little Harbor, Aug. 31, 1940; also of *P. Harveyi* Bail. *v. Olneyi* (Harv.) Collins, coll. Jennie L. S. Simpson, in the wash at Nobska Point, Sept. 9, 1942.

The alga was found by Börgesen growing at a depth of 5 fathoms on *Griffithsia globulifera* Harv., near Buck Island, St. Croix, Virgin Islands. It was again collected by Taylor, growing in the cell wall of old specimens of Bryopsis washed ashore in Rockly Bay, Tobago Island, Br. West Indies. Thus the alga occurs in the sublittoral region and probably also at intertidal levels.

Distribution: West Indies, Atlantic Coast of North America.

Börgesen, 1920, pp. 418–19, figure 400; Taylor, 1942, pp. 15–16.

In the specimens collected at Woods Hole in 1942, while observing the cellulose tubercles, the presence of very delicate setae was noticed for the first time in this species. Börgesen's figures, especially 400c and d, agree with the appearance of the tubercles seen in the above specimens, but he was of the opinion that the structures in question on his plants were small cells. He remarks that now and then five to six or more narrow bodies, lying above each other, are present in them. He also says these bodies are filled with starch and leaves it to further observations on living specimens, as he had studied only dry ones, to add information about them. Though Börgesen does not refer to the cellulose tubercles,

---

PLATE IV

Ectochaete vagans (Börgesen) *comb. nov.*

On *Polysiphonia Harveyi* Bail. *v. Olneyi* (Harv.) Collins

FIGURE 1. Habit of endophyte within the host wall; no tubercles or setae seen, × 75.

FIGURE 2. Thallus showing fusions in the center and lacking tubercles and setae; two open sporangia seen, × 352.

FIGURE 3. Two young plants; a tubercle seen at *a*, × 352.

FIGURE 4. Lateral view of two filaments within the host wall; setae with conical incrassate bases, × 749.

FIGURE 5. Germling showing the germination tube dividing into two cells and the empty zoöspore, × 1649.

FIGURE 6. Two-celled germling embedded in the host wall with the empty zoöspore on the surface of the host, × 666.

FIGURE 7. Three-celled germling, × 465.

On *Polysiphonia flexicaulis* (Harv.) Collins

FIGURE 8. Plant showing reticulate habit, tubercles, sporangia and empty sporangia, × 273.

FIGURE 9. Filament showing three sporangia. Two with the opening in the center of the tubercle, × 431.

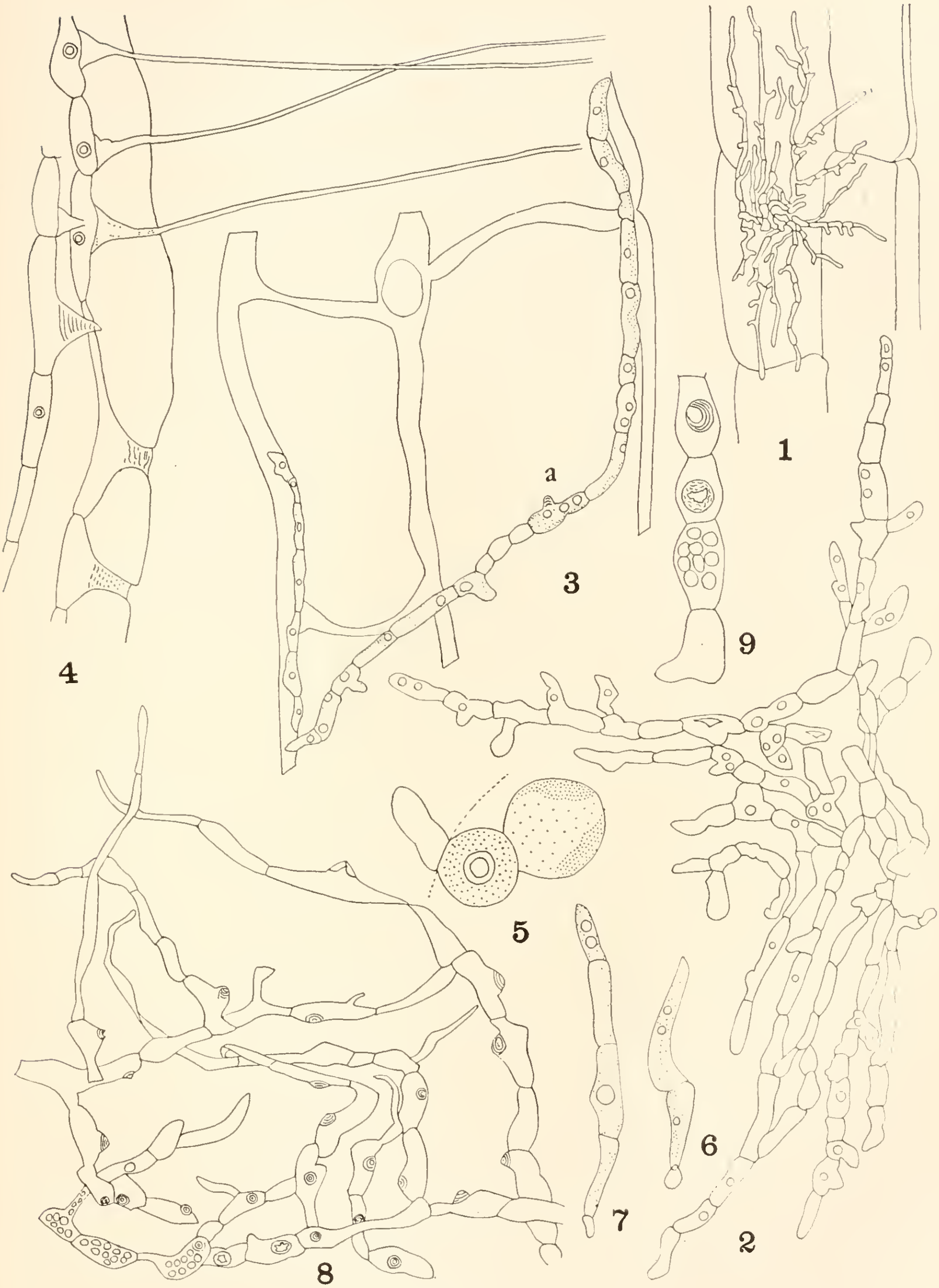

1
a
3
4
9
2
5
6
7
8

PLATE IV

the figures cited above probably depict these structures, considered by him as elevations of cells cut off by a cell wall.

In the living examples the elevations or outgrowths of cells are distinct from the cellulose tubercles. The former show the presence of large amounts of starch, becoming black like the rest of the cell contents when treated with Schultz's solution. Occasionally the apex of an outgrowth develops into a cellulose tubercle (Plate V, figures 12 and 13), which takes on a violet color with the above reagent. The tubercles that also occur directly on the cells do not contain cell contents, but consist entirely of wall substance.

The presence of setae in the collection from Woods Hole leaves little doubt that the conical tubercles are the persistent bases of the setae, while the dome-shaped and peg-like ones appear to be structures that are either *sui generis*, or setae arrested in their development. In common with the setae of other endophytic Chaetophoraceae, those of the present alga presumably function in creating a large surface of contact with the medium external to the host; the persistent bases of the setae as well as the other tubercles very likely subserve the same rôle.

The setigerous character of the species necessitates its transfer from Endoderma Lagerh. to Ectochaete (Huber) Wille.

The habit of Phaeophila and Ectochaete may be looked upon as reduced (specialized) and being derived from a heterotrichous habit in which the erect system has been replaced by setae, or it may be considered relatively primitive, and as evolving from a simpler entirely procumbent habit (Fritsch, 1942, p. 401). Erect filaments developing instead of setae in cultures of these two genera may have not only physiological but also phylogenetic meaning (Huber, 1892a, p. 333).

The setae of Ectochaete leptochaete, E. vagans *and* Phaeophila Engleri

A seta continuous with the lumen of the supporting cell is said to be characteristic of the genus Ectochaete (Huber, 1892a, p. 331, figure 5; Printz, 1927, p. 194). In the present cultures of *E. leptochaete* the setae show open bases in favorable examples; in others the bases look solid, the setae appearing to take

---

PLATE V

Ectochaete vagans (Börgesen) *comb. nov.*

On *Polysiphonia Harveyi* Bail. *v. Olneyi* (Harv.) Collins

FIGURES 1–3. Filaments with tubercles on the wall; in Figure 1 the broken line representing the host wall × 1000.

FIGURES 4–5. Cells showing the bases of the setae, × 1000.

FIGURE 6. At *a*, seta with an opaque base, showing the lumen in the upper part; at *b*, a peg-like tubercle on the wall, × 1000.

FIGURES 7–8. Long terminal cells of two filaments, × 1000.

FIGURE 9. Filament with two sporangia, one showing a tubercle, × 1000.

FIGURES 10–11. Germlings showing the empty zoöspore outside the host wall and the germination tube within; Figure 10 × 764; Figure 11 × 1000.

FIGURE 12. Filament showing a cell bearing a process with an apical thickening of the wall at *a*, and a peg-like tubercle on the wall at *b*, × 1000.

On *Polysiphonia flexicaulis* (Harv.) Collins

FIGURE 13. Filament showing a cell process with an apical thickening of the wall at *a*, and an open sporangium at *b*, × 575.

FIGURE 14. Three Sporangia, each with a tubercle, × 575.

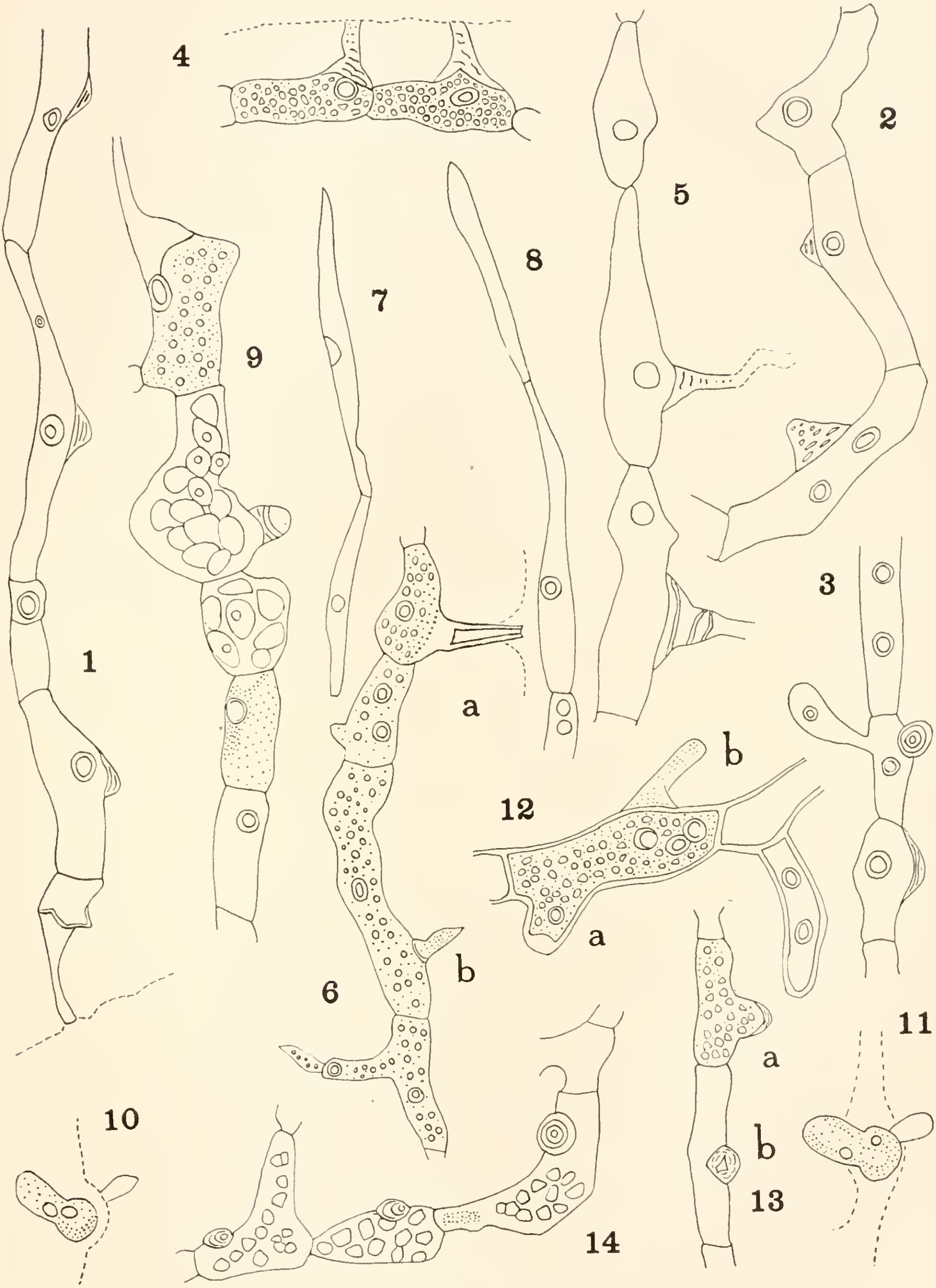

4
2
5
8
7
9
1
a
3
b
12
a
6
b
a
11
10
b
13
14

PLATE V

their origin from the cell wall, however the lumen is clear along the rest of the length of the seta and it contains granules especially at the apical part. A rather similar condition is present in the genus Chaetosphaeridium.

Klebahn (1892, p. 269, Plate IV) considers the setae of *C. minus* Hansg. (*C. Pringsheimii* Klebh.; *Aphanochaete globosa* [Nordst.] Wolle *var. minor* Hansg., cf. West, 1904, p. 182–83) as agreeing in structure with those of Coleochaete, and his figures show continuity of the lumen from cell to seta, while Möbius (1892, p. 104, figure 8) says with reference to the setae of the above alga (for synonymy cf. Hazen 1902, p. 228) ". . . sondern hier findet sich eben nur die Kommunikation durch Verdickung der Membran sehr verengt." Huber (*l. c.*, pp. 338–39) pointed out that the setae of *Chaetosphaeridium globosum* (Nordst.) Klebh. (*Aphanochaete* var. *a globosa* Hansg.) are transitional between tubular and solid setae, since their lumina are extremely narrow with protoplasm represented by only a few granules, and Oltmanns (1922, I, p. 303) cites the same for the species. On the other hand, Wille (1909, p. 100), Heering (1914, p. 144), Printz (1927, p. 233) attribute solid, homogeneous, tegumentary setae to the genus, and Fritsch (1935, p. 286) considers that setae of this type characterize the family. The lumen is so greatly reduced in the genus that both these interpretations exist.

The difficulty of seeing the passage between the seta and its supporting cell, because of the thickness of the wall and the narrowness of the lumen at the base of the seta, may give rise to the view that the setae are tegumentary in *E. leptochaete*, but it is possible in some cases to see clearly the passage from the cell into the seta, and this confirms Huber's description of them.

The setae of *Ectochaete vagans* and *Phaeophila Engleri* are comparable with those of *Acrochaete repens* Prings. At Woods Hole, in certain cells of Acrochaete the lumen of the seta could be seen connected with that of the cell (Plate VI, figure 1, *a* and *c*) while in most cases the seta is refractive and cannot be viewed in optical section (Plate VI, figure 1, *b* and figure 4); in a few instances it was found also that a septum may arise at the base of a seta, separating it from the cell, and in these examples the setae had been shed, breaking a little above their bases (Plate VI, figure 3). Huber (*l.c.*, p. 328, figure 3, b) considers such septa as secondary developments since the setae lack nuclei of their own.

The opaque conical base of the seta in *Ectochaete vagans* similarily cannot be seen in optical section, but since a lumen is visible at times above the base of the seta (Plate V, figure 6*a*) a connection probably exists between seta and cell as in Acrochaete.

The basally open and the basally septate setae of *Phaeophila Engleri* agree with the corresponding setae of Acrochaete, and as in the latter the formation of a basal septum in certain of its setae is presumably secondary.

It is of interest to note that the cells of Acrochaete may have cellulose tubercles as in both the species compared with it above, but in it invariably found at the apices of cell processes (Plate VI, figure 2).

LEPTOSIREAE

Entocladia Reinke, 1879. Algae forming discs in the cell wall, immersed in the mucilage of the host, penetrating the tissues of the host, or found in the cuticular layer of Coelenterata and Bryozoa and in the shells of molluscs; thallus of radiating filaments often subparenchymatously congested in the center; hairs and setae absent; cells cylindrical to isodiametric, sometimes irregularly swollen, uninucleate, with a parietal chloroplast and one to several pyrenoids; sporangia appearing in large numbers, sometimes slightly larger than vegetative cells, provided with a papilla-like or short, tubular beak, producing 8–28 usually quadriflagellate zoöspores and exceptionally biflagellate zoöspores and isogametes; germination of zoöspore endophytic or epiphytic in type.

Entocladia testarum Kylin. Alga inhabiting the superficial layers of the dead shells of molluscs, in shallow water and salt marshes; thallus forming a more or less spherical mass of interlacing filaments; internal filaments partly fused, consisting of round to oval or irregular shaped cells 7.1–10.58 μ diam. and of cylindrical cells 3.52 μ diam., 3–8 times as long; in the intermediate region of the thallus one-celled, oval, decumbent branchlets present, with a diameter of 9.4 μ; outer filaments of the semispherical mass free, with cylindrical cells about 3.53 μ diam., 3–8 times as long, bearing one to several decumbent or vertical, papilla-like processes 3.53–5.88 μ diam.; tips of outer filaments bending upwards towards the surface of the shell; cells with a parietal, plate-like, sometimes perforate chloroplast; starch grains often numerous, making the chloroplast appear reticulate; pyrenoids 1–5, usually 2 or 3; sporangial cells cylindrical to club-shaped or round 7.06–12.94 μ diam., and neck 4.71–5.88 μ diam., 7.1–9.41 μ long; zoöspores 4–14 in each, 2.35–4.1 μ diam., twice as long, pear-shaped, quadriflagellate, with flagella equalling the cells in length, with an eye-spot, a chloroplast, a pyrenoid, and starch grains; germination of the epiphytic type, not involving the formation of a germination tube.

Woods Hole, Massachusetts: Rich growth in the empty shells of *Mya arenaria* L. along with traces of *Gomontia polyrhiza* and *Phaeophila Engleri*, Penzance salt marsh coll. Jennie L. S. Simpson, August 29, 1942.

Europe: Kristineberg, Swedish west coast, very abundant on dead *Mya arenaria* shells, Kylin.

Distribution: Baltic Sea; Atlantic coast of North America.

Kylin, 1935. pp. 197–201, figures 5 A–R; 6 A–F.

Kylin gives an account of the characteristics of the alga in cultures, both isolated and on shells. In the former case spherical masses of filaments were formed with cells larger than in nature, and with the length of sporangial necks reaching twice the diameter of the sporangia (Kylin 1935, figure 5 P and R) that is, a little longer than when within the shell. When a culture was grown on *Mya arenaria* shells, some of the filaments emerged from it, and these were 10–15 μ wide while the immersed filaments were 5–10 μ wide; sporangia were obtained under both conditions.

Kylin points out (*l. c.*, p. 203) that the species is difficult to distinguish from *E. tenuis* Kylin and that the two usually are found together, with Gomontia and Phaeophila; but he mentions that the cells are narrower and longer in *E. tenuis*, (the measurements being 3–5 μ diam., 8–20 times long in the young cells and 5–8 μ diam., 4–8 times long in older ones). Since no cells in *E. testarum* as met with at Woods Hole, in agreement with Kylin's description, exceed the length of 8 times their diameter, and because of the characteristic cushion habit with numerous decumbent or erect branchlets, cell processes, and branch tips, found in nature in the present specimens, but seen by Kylin only in his cultures, the species appears to be singular and unmistakable. Moreover a definite difference between the latter two species is to be seen in their germlings (*l. c.*, p. 204) as *E. testarum*, unlike *E. tenuis*, lacks a germination tube.

Regarding the phylogenetic significance of the plagiotropic dendronema or nematoparenchyma (terms *ex* Schussnig, 1938) under which types the Leptosireae come, Fritsch postulates: ". . . the first step in the evolution of the heterotrichous habit may have been a branched creeping filament or expanse attached throughout to the substratum, . . . a possibility not incompatible with the existence of prostrate types that have arisen by reduction from a heterotrichous filament."

ULVELLEAE

Ochlochaete Thwaites, 1849. Plants epiphytic on marine phanerogams and algae, or creeping on stones, shells and debris, green or olive-green in color; thallus filamentous, but more or less pseudoparenchymatous from the beginning; branches subdichotomous, either (*O. ferox*, *O. lentiformis*, *O. gratulans*) superposed

---

PLATE VI

Acrochaete repens Prings.

FIGURE 1. Filament showing at *a* and *c* continuity of the lumen between cell and seta at *b* the opaque base of a seta, × 528.

FIGURE 2. Filament with vertical cell processes, each with a tubercle at the apex, × 329.

FIGURE 3. A cell showing a secondary septum between seta and cell process, × 947.

FIGURE 4. Cell with seta showing lumen above and opaque base below, × 528.

Entocladia testarum Kylin
From Mya

FIGURE 5. Superficial cells of the thallus showing horizontal filaments, erect processes and unicellular branches, × 991.

FIGURE 6. Terminal cell with two erect processes, × 991.

FIGURE 7. Filament with a decumbent apex, a lateral process, a decumbent lateral process at *a*, and a vertical process at *b*, × 1142.

FIGURE 8. A horizontal filament from the intermediate region of the thallus showing a unicellular decumbent branchlet, × 1142.

FIGURE 9. Filament showing two uninucleate cells and the decumbent tip of a process, × 1142.

FIGURE 10. Unicellular branch showing nucleus, chloroplast and pyrenoid, × 1273.

FIGURE 11–13. Intercalary sporangia showing necks, × 1142.

Ochlochaete lentiformis Huber

FIGURES 14–15. Young discs from Busycon, × 529.

FIGURE 16. Cells from a disc growing on a stone showing arrangement in three fused layers, × 529.

FIGURE 17. Cells from a disc growing on a stone showing two fused layers, × 529.

FIGURE 18. Marginal cell of a young disc showing the basally open seta, from Busycon, × 259.

FIGURE 19. Seta with the lumen narrowed at the base, from a stone, × 1142.

FIGURE 20. Two-celled germling from Busycon, × 2283.

FIGURE 21. Transverse section of thallus showing sporangia, one of them with zoöspores, × 800, figure from Huber, 1892b.

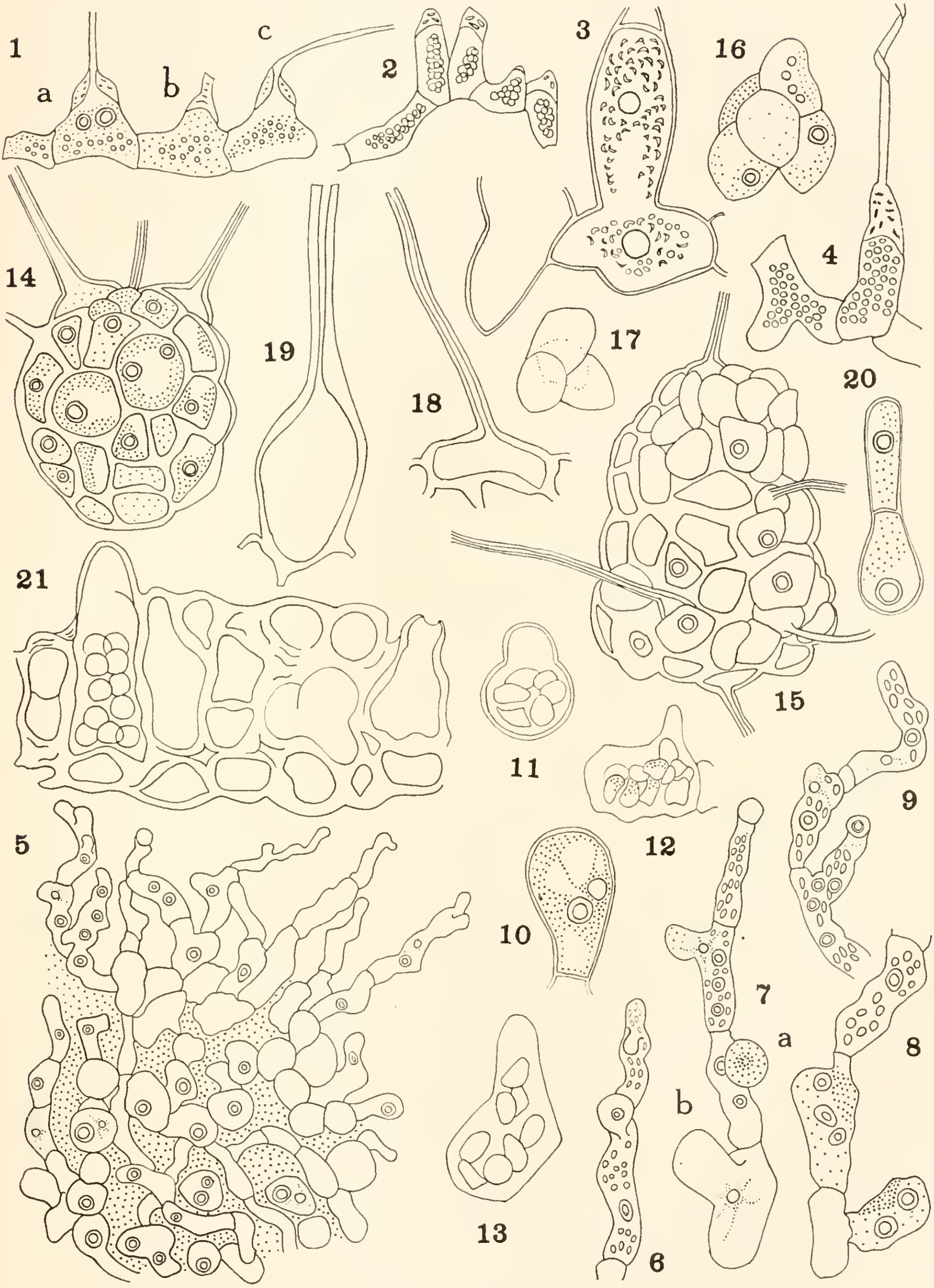

1
a
b
c
2
3
16
4
14
19
18
17
20
15
21
11
12
9
5
10
7
a
8
b
13
6

PLATE VI

and completely fused, resulting in a 2–3-stromatic disc, or (*O. hystrix*) in one plane and united only in the centre of the thallus; some of the cells bearing a seta; seta thick walled, firm, straight, showing sparse contents, with lumen continuous with that of the supporting cell; cells round to rectangular, uninucleate; chloroplast parietal, plate-like, with usually one and sometimes 2–3 pyrenoids, often with numerous starch grains; reproduction by 4-flagellate zoöspores, produced in large numbers in sporangia provided with a short neck; zoöspores ovoid with a hyaline beak and a pyrenoid, escaping in a mass and expelled with force; zoöspore germinating by growing at right angles to its long axis, *i. e.* epiphytic in type.

Ochlochaete lentiformis Huber. Alga forming green specks on the surface of stones, shells and debris; disc 51–831 μ in diam., more or less circular, pulvinate, consisting of filaments fused into a firm tissue, without intercellular spaces, 2–3-stromatic in the center, monostromatic at the periphery; short erect branches of one or two cells in length arising near the margin of the disc, making it polystromatic; margin of disc truncate, or showing free ends of filaments to a depth of 1–2 cells; cells often exhibiting a radial arrangement in the disc, 5.1–12.75 μ diam., sometimes up to 22.95 μ diam., some of the superficial cells of the disc at times only 3.5 μ diam.; cells at the margin in old discs round or oval with the radial axis longer, in young discs as above and also sometimes when oval with the long axis in the tangential direction; superficial and marginal cells often bearing a seta; setae 2.35–3.82 μ diam. near the base, 0.85–1.8 μ near the tip, about 0.5–1 mm. long; lumen of seta and supporting cell confluent; chloroplasts plate-like, with 1–3 pyrenoids; sporangia with a short neck, forming about 16 zoöspores expelled together explosively, germinating without producing a germination tube.

Woods Hole, Massachusetts: On old dead shell of *Busycon carica* Gmelin, Great Harbor, coll. W. J. Gilbert, July 30, 1941; on dead shells of *Polynices duplicata* (Say), on dead shells of *Anomia simplex* D'Orbigny, on broken bits of porcelain, on white pebbles, all at the Spindle, August 26, 1942.

Europe: Croisic, Bay of Biscay, abundant on pieces of porcelain, old pipes and glass, Huber, September 1891.

Distribution: Atlantic coasts of France and North America.

Huber, 1892b, pp. 296–97, Plate XI, figures 1–3; Wille, 1909, p. 88; Printz, 1927, p. 211; Hamel, 1930, pp. 44 and 46.

This alga may pass for *Protoderma marinum* Reinke, associated with which it was seen on stones, pieces of porcelain and shells in the present collections. They are alike in forming pseudoparenchymatous discs several layers in thickness, but they are distinguished by the presence or absence of setae. When setae are few in *O. lentiformis* it can be distinguished easily by the size of its cells, for in *P. marinum* the cell diameter is about a half of that of the former and is 3.5–7.65 μ, some of the central cells occasionally reaching up to 12 μ. The central cells in both are more or less round though they may be isodiametric-angular in

Protoderma marinum. The marginal cells are round or oval in both, but sometimes oblong in *P. marinum.*

O. ferox Huber (*l. c.*, pp. 292–93, Plate X, figures 1–10) is known for Massachusetts (Collins, 1909, p. 288; Taylor, 1937, p. 55). It differs from the present species in choice of substratum, being epiphytic on Cladophora, Chaetomorpha and Zostera, as well as by the larger size of its thallus and cells, and the presence of setae in greater numbers.

Fritsch (1935, p. 260) considers the upper layers as possibly formed by short erect adpressed branches, while Huber described them as arising from superposed, horizontally growing branches. Both interpretations are tenable, since the branches are not long enough to see whether they remain erect or grow parallel to the surface of the disc. The habit of Ochlochaete, with little doubt, represents the incipient, plagio-orthotropic crust or cushion which finds its best expression among the Chaetophorales, according to Fritsch (1942, p. 401), in Pseudopringsheimia.

The genus has been reported to have only a single pyrenoid in each cell, except by Hylmö (1916, p. 29) who found two in the longer cells; the present examples of *O. lentiformis* have frequently 2–3 pyrenoids to a cell.

Chaetosphaeridiaceae

Diplochaete solitaria Collins. This epiphytic alga was found at Woods Hole, adhering to a filament of *Polysiphonia Harveyi* Bail. *v. Olneyi* (Harv.) Collins collected in the wash at Nobska Point, on September 10, 1942. In cell measurements, thickness of cell wall and characters of the solid setae, the specimen falls completely within the description given by Collins. A pyrenoid was not seen; its presence was doubted when the alga was first described. Only two individuals were seen in the present instance and the alga appears to be rare.

Diplochaete remains a monotypic genus as when originally described, since Polychaetophora W. et G. S. West, which was united with Diplochaete by Collins (1909, p. 278), has been reestablished, and the genus Oligochaetophora created for *P. simplex* G. S. West, a unicellular epiphytic form like *D. solitaria* but found in fresh water (West, 1911, pp. 88–89).

Distribution: West Indies (Jamaica, on *Laurencia obtusa*); Massachusetts as above.

Collins, 1901, p. 242; 1909, p. 277–78, figure 99; Wille, 1909, p. 103; Printz, 1927, p. 231, figure 178.

LITERATURE CITED

Batters, E. A. L., 1902. A catalogue of the British marine algae. *Jour. Bot.*, **40**: 1–107, Supplement.

Börgesen, F., 1915–20. Marine Algae of the Danish West Indies, II. *Dansk Bot. Arkiv*, **3**: 1–504.

Bornet, E., and C. Flahault, 1888. Note sur deux nouveaux genres d'algues perforantes. *Jour. de Bot.*, **2**: 161–165.

Bornet, E., and C. Flahault, 1889. Sur quelques plantes vivant dans le test calcaire des mollusques. *Bull. Soc. Bot. France*, **36**: 147–174.

Collins, F. S., 1901. The algae of Jamaica. *Proc. Amer. Acad. Arts and Sci.*, **37** (9): 231–270.

Collins, F. S., 1909. The green algae of North America. *Tufts Coll. Stud.*, **2** (3): 79–480.

Feldmann, J., 1937. Les algues marines de la côte des Albères II, Chlorophyceae. *Rev. Algol.*, **9** (3–4): 173–241.

Fritsch, F. E., 1935. The Structure and Reproduction of the Algae. Vol. I. Cambridge, Eng.

Fritsch, F. E., 1942. Studies in the comparative morphology of the algae; I. *Ann. Bot.*, new ser., **6** (23): 396–412.

Hamel, G., 1930. Chlorophycées des côtes françaises. *Rev. Algol.*, **5**: 1–54.

Hansgirg, A., 1892. Vorläufige Bermerkungen über die Algengattungen Ochlochaete Crn. und Phaeophila Hauck. *Österr. Bot. Zeitschr.*, **42** (6): 199–201.

Hazen, T. E., 1902. The Ulothricaceae and Chaetophoraceae of the United States. *Mem. Torr. Bot. Club*, **11**: 135–250.

Heering, W., 1914. Chlorophyceae III, *in* Pascher, A., Süsswasserflora Deutschlands, Österreichs und der Schweiz, **6**: I–IV, 1–250.

Huber, M. J., 1892*a*. Observations sur la valeur morphologique et histologique des poils et des soies dans les Chaetophorées. *Jour. de Bot.*, **6**: 321–41.

Huber, M. J., 1892b. Contributions à la connaissance des Chaetophorees epiphytes et endophytes et de leur affinités. *Ann. Sci. Nat.*, Bot., VII, **16**: 265–359.

Hylmö, D. E., 1916. Studien uber die marinen Grünalgen der Gegend von Malmö. *Arkiv för Bot.*, **14** (15): 1–55.

Klebahn, H., 1892. Chaetosphaeridium Pringsheimii, novum genus et nova species algarum Chlorophycearum aquae dulcis. *Jahrb. wiss. Bot.*, **24**: 268–282.

Kylin, H., 1935. Über einige kalkborhende Chlorophyceen. *Kungl. Fysiogr. Sällsk. i Lund*, Forhandl., **5** (19): 186–204.

Lakowitz, K., 1929. Die Algenflora der gesamten Ostsee. 747 pp. Danzig.

Migula, W., 1907. Algen. *In* Thomé, Flora von Deutschland, Österreich und der Schweiz, VIB, **2** (1B): 513–917.

Möbius, M., 1892. Morphologie der haarartigen Organe bei den Algen. *Biol. Centralbl.*, **12** (4): 97–108.

Nadson, G. A., 1927. Die kalkbohrende Algen des Schwarzen Meeres. *Arch. Russ. Protistol.*, **6**: 147–53.

Newton, L. B., 1931. A Handbook of the British Seaweeds. British Mus. Nat. Hist. 478 pp. London.

Oltmanns, F., 1922. Morphologie und Biologie der Algen I: 1–459. Jena.

Pratt, H. S., 1935. A Manual of the Common Invertebrate Animals exclusive of Insects. XVIII + 854 pp. Philadelphia.

Printz, H., 1927. Chlorophyceae (nebst Conjugatae, Heterocontae und Charophyta). *In* Engler, A., and K. Prantl, *Die natürl. Pflanzenfam.*, *2. Aufl.*, **3**: 1–463.

Reinke, J., 1889. Algenflora der westlichen Ostsee deutschen Anteils. VI. Bericht der Komm. zur wissensch. Unters. der deutsch. Meere. 101 pp. Berlin.

Schussnig, B., 1938. Vergleichende Morphologie der niederen Pflanzen I: I–VIII, 1–382. Berlin.

Taylor, W. R., 1937. Marine Algae of the northeastern coast of North America. IX + 427 pp. Ann Arbor.

Taylor, W. R., 1942. Caribbean Marine Algae of the Allan Hancock Expedition, 1939. *Univ. South. California Publ. Allan Hancock Atlantic Exped.*, Report **2**: 1–193.

Thivy, F., 1942. A new species of Ectochaete (Huber) Wille from Woods Hole, Massachusetts. *Biol. Bull.*, **83**: 97–110.

West, G. S., 1904. British Freshwater Algae. XV + 372 pp. Cambridge, Eng.

West, G. S., 1911. Algological Notes. *Jour. Bot.*, **49**: 82–89.

Wille, N., 1909. Conjugatae und Chlorophyceae in *Die natürl. Pflanzenfam.*, *Nachtr. z.* I, **2**: 1–284.

POLARIZATION, KINETOCHORE MOVEMENTS, AND BIVALENT STRUCTURE IN THE MEIOSIS OF MALE MANTIDS

SALLY HUGHES-SCHRADER

(*Department of Zoology, Columbia University*)

INTRODUCTION

The pioneer studies of mantid cytology of Giardina (1897), Oguma (1921) and King (1931) were concerned primarily in establishing the chromosome complement and the existence of the X_1X_2Y, ♂, and $X_1X_1X_2X_2$, ♀, sex chromosome mechanism. The meiotic bivalents were found to conform to the usual orthopteran types and few or no data were given on the prophase behavior of any except the sex chromosomes. The later papers of Williams (1938) and Erazi (1940), while in general supporting the earlier conclusions, are based on inadequate material and analysis. Erazi's report of an XY or XX sex chromosome complement in the male of *Empusa pennicornis* should be checked on more extensive material.

The recent studies of White (1938, 1941) have resulted not only in his beautiful analysis of the compound sex chromosome complex, and the discovery of an XO ♂, XX ♀ sex chromosome mechanism in many species, but have also disclosed in the meiosis of male mantids three other problems of major cytological interest. First is the nature of the bouquet stage. The occurrence of two separate polarizations of the chromosomes at different periods of the prophase,—the second of which takes place at pachytene—offers an exceptionally favorable opportunity for an analysis of bouquet formation. The second problem is posed by the complicated series of kinetochore movements. In most mantid species the spindle forms in late pachytene, whereupon homologous kinetochores move suddenly apart toward opposite poles forcibly stretching open the bivalents in the developing spindle. This unique movement is followed by the re-approach of the homologous kinetochores as the chromosomes again contract; only then do the bivalents move to their final position in the metaphase plate, following which the real anaphasic movement is initiated. The third problem—the relation of chiasmata to bivalent structure—is presented by the variation among different species in the form of the bivalents during the stretching process and at first metaphase. In Callimantis, where the stretch phenomenon is absent, the bivalents retain the parallel association of their homologous chromosomes, except for a localized separation at the kinetochore region, until anaphase. The cytological evidence for the complete absence of visible chiasmata at all stages in these bivalents is unequivocally clear (White, 1938; Hughes-Schrader, 1943). In all the other species investigated by White (1941) the pre-metaphase stretching of the bivalents discloses terminal connections between their homologous chromosomes suggestive of previous chiasma formation.

Obviously the amazing range of chromosome and more specifically kinetochore movement, and in type of bivalent structure indicated above afford data which bear significantly on many problems of the mitotic mechanism. Until an experimental analysis becomes possible, the comparative study of these phenomena in many related species offers the best approach to the problems they pose. The present report covers five species, in one of which are found two distinct types of prophase behavior correlated with different geographic distribution. The cytological data are presented separately for each species,—followed by a comparative study of the problems outlined above as clarified by the variations presented in the different species.

Material and Methods

Males of the following four species were collected on Barro Colorado Island, Panama Canal Zone, during December of 1939 and 1940: *Liturgousa annulipes* Serv.,—six pre-adult nymphs, two adults; *Stagmomantis carolina* Johann,—two adults; *Angela guianensis* Rehn,—one adult; and *Choeradodis rhombicollis* Latreille,—two adults. *Stagmomantis carolina* was also collected in the region of Onancock, Virginia, in July and August 1941 and 1942; fifteen pre-adult and two adult males were used in the present study. Also from Onancock is the material of *Paratenodera sinensis* Saus.,—comprising seven pre-adult males. My experience confirms White's (1941) report that the pre-adult male offers the most extensive range of stages in spermatogenesis. It is a pleasure to thank Dr. James A. G. Rehn of the Academy of Natural Sciences of Philadelphia for the identification of all of the specimens, with the exception of the Paratenodera.

The fixatives of Sanfelice, and Bouin as modified by Allen and Bauer, were used exclusively. Material was sectioned at from 8 to 12 micra and variously stained in iron haematoxylin, Smith's modification of Newton's gentian violet, La Cour's chromic acid gentian violet, and Feulgen. Counterstaining with erythrosin after gentian violet and with light green after Feulgen was useful for spindle and nuclear membrane differentiation.

Cytological Data

The three main problems—(1) the second polarization or bouquet stage, (2) kinetochore movements, and (3) bivalent structure and chiasmata, will be considered separately under the different species. Early prophase, prior to pachytene, is remarkably uniform in all and a few words here will apply to all the species studied. Leptotene and zygotene stages fix and stain poorly, and no detailed analysis of them has been attempted. The major features of chromosome behavior can, however, be established. Leptotene is characterized by a typical bouquet formation in which the ends of all the autosomes are aggregated on a restricted region of the nuclear membrane immediately underlying the division center,—while the bodies of the chromosomes extend through the nucleus in loops. The sex chromosomes are also polarized. In Liturgousa and Angela the single X undergoes a typical conflexion and its closely appressed ends are aggregated with those of the autosomes. The behavior of the multiple sex chromosomes of Stagmomantis, Choeradodis, and Paratenodera is less open to analysis, but in Paratenodera at least it can be shown that the ends of all three sex chromosomes are involved in the polarization. The bouquet formation persists through zygotene and terminates at different times of the prophase in the different species, but usually during early pachytene. By this time the division center is no longer visibly differentiated from the general cytoplasm.

Stagmomantis carolina *from Virginia*

The diploid complement of the male of this species is 27,—12 pairs of autosomes plus X_1, X_2, and Y. The chromosomes are figured and described by King (1931); I shall therefore confine my account to those aspects of the meiotic prophase bearing on the problems outlined.

I. Second polarization

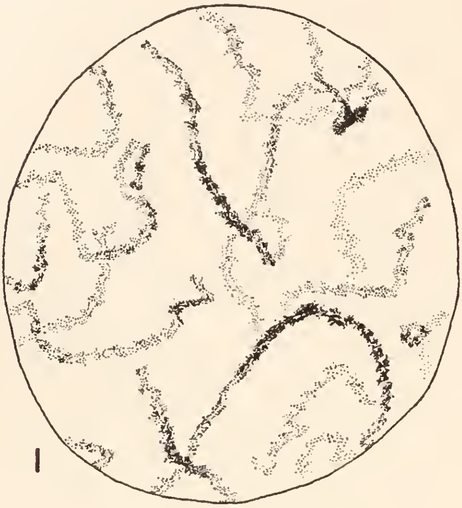
With the cessation of polarization in early pachytene the chromosome ends assume a random distribution in the nucleus, and a completely unoriented stage ensues (Fig. 1). By mid pachytene the division centers again become visible as asters form around them. Thereupon the chromosome ends once more orient actively and aggregate on the nuclear membrane underlying the centers. For the second time, therefore, in this prophase, a bouquet formation is brought about. Two division centers are now involved, however, and this results in variation in the pattern of polarization. Both ends of a bivalent may move to the same pole, or one end may go to each pole stretching the body of the bivalent between them. A double bouquet results—with the ends of the bivalents variously distributed at the two centers (Figs. 2 and 3). The centers are usually already on opposite sides of the nucleus when they first become visible, but occasionally the timing varies and the two poles may lie no more than 90° apart. In such cases, as the centers continue to move toward opposite sides of the nucleus, the grouped chromosome ends follow them on the inside of the membrane, for by late pachytene the two bouquets are invariably some 180° apart. [This precocious activity of the centers and consequent shifting of the aggregated chromosome ends is more common in the Barro Colorado form of Stagmomantis and is illustrated in Figures 12 and 13.] Such a sliding movement of the chromosome ends along the membrane suggests that no real fusion or firm cementing of the two is involved,—although after appropriate staining it is possible to demonstrate that the terminal chromomeres of each bivalent are closely appressed against the nuclear membrane (Fig. 4). The attenuation of the ends of the bivalents might suggest that they are under tension,—but since it is equally apparent in bivalents looped at one pole as in those stretched between two, this impression is misleading. The apparent attenuation more probably stems from the fact that as the gyres of the chromosomes increase in diameter and decrease in number —progressively from the kinetochore distally—the ends are the last to be affected.

II. Kinetochore movements

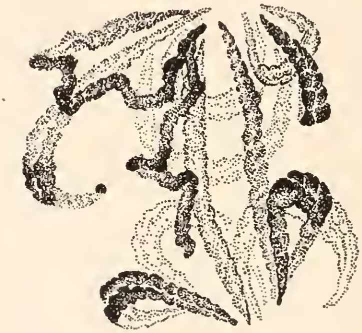
a. Kinetochore separation, spindle formation, and stretch.

The nuclear membrane disappears early, while the nucleus is still in a late pachytene stage. The spindle forms immediately, filling the entire nuclear area, and undergoes a rapid elongation. Coincidently the bivalents are released from their polarized condition. Thereupon their ends appear flaccid and evince no further specific movement.

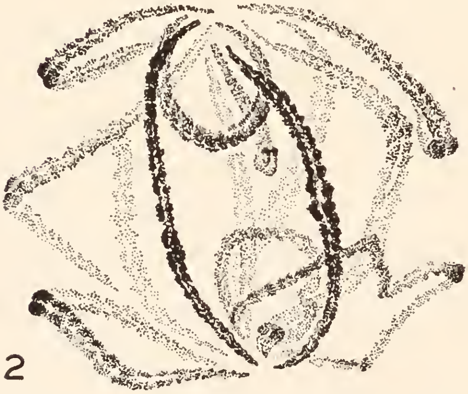
The two kinetochores of each bivalent now separate sharply and orient toward opposite poles (Fig. 5). This movement occurs while the bivalents are scattered throughout the nuclear area, their position reflecting the grouping of the preceding polarization (Figs. 5, 6, and 7). Bivalents lying close to one pole (upper right, Fig. 6) may show as extreme an initial separation of kinetochores as those in the equator.



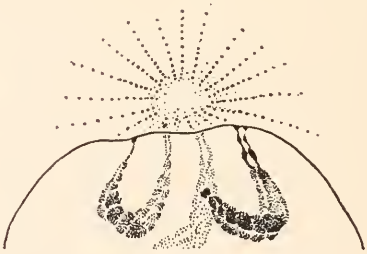
1



3



2



4

(All drawings made with camera lucida at table level with Zeiss 2 mm., n.a. 1.3, obj. and 20 × oc.; enlarged with pantograph. Magnification as reproduced 2700 ×.)

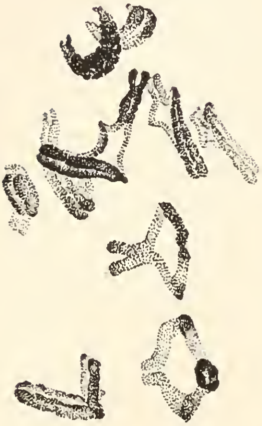
FIGURES 1 TO 4. *Stagmomantis carolina*, Virginia.

FIGURE 1. Non-polarized early pachytene; upper level only drawn; ends distributed at random. Feulgen.

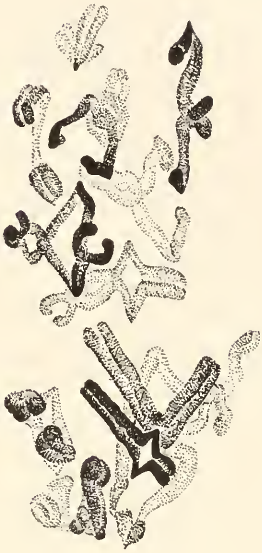
FIGURE 2. Second polarization, in mid-pachytene. Feulgen; intact membrane and division centers at poles unstained.

FIGURE 3. Later stage in second polarization. Feulgen.

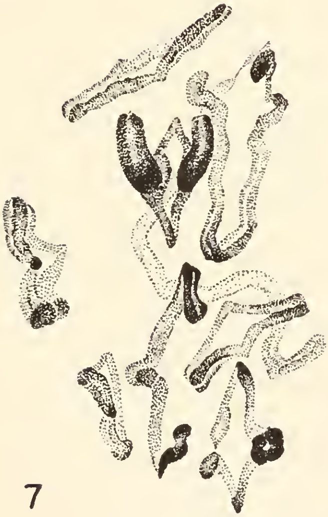
FIGURE 4. Detail of same after gentian violet and erythrosin; terminal chromomeres applied to membrane under center.



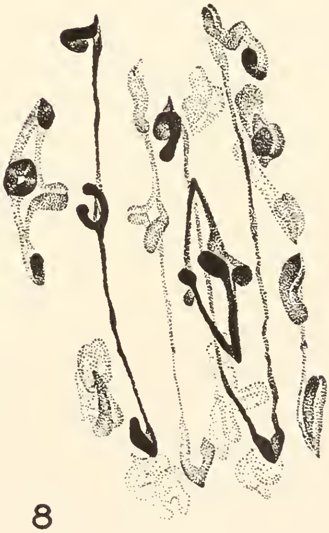
5



6



7



8

FIGURES 5 TO 8. *Stagmomantis carolina*, Virginia.

FIGURE 5. Early stretch; membrane gone, upper bivalents still polarized, other bivalents opening as kinetochores orient to poles. Feulgen.

FIGURES 6 AND 7. Early stretch; asynchrony of bivalents; secondary loci of separation between homologues; spindle elongation complete. Feulgen.

FIGURE 8. Mid-stretch; some bivalents not yet opened; open cross in several bivalents here and in Figure 9. Feulgen.

The events just described—break down of nuclear membrane, formation and elongation of spindle, separation and polar orientation of homologous kinetochores—take place with great rapidity as is shown by the rarity of these stages compared with those which precede and follow them. Other evidence supports this. Thus, cells in which the nuclear membrane has but just collapsed, as shown by the persistent polarization of some of the bivalents (top, Fig. 5), already show continuous spindle fibers between the centers, and a marked increase in intercenter distance over immediately preceding stages with membrane intact. Again, in stages such as Figure 6, whose closeness to Figure 5 is attested by evidence in the distribution of the chromosomes of their previous polarization, the spindle has already attained almost its maximum length. Indeed, the elongation of the spindle is always completed before all of the bivalents have been stretched open by the poleward movement of their kinetochores. [Measurements of spindle length at these and later stages are given in Table 1; a consideration of the role of spindle elongation in the kinetochore movement is reserved for the discussion—in comparison with data from the other species studied.]

The movement of homologous kinetochores toward opposite poles continues—often to an extreme degree. Not infrequently two thirds or more of the total spindle length may be traversed by the separating kinetochores of a given bivalent. In the process, the homologous chromosomes of each bivalent are stretched and pulled apart, retaining only terminal or subterminal connections in one or both arms (Figs. 8 and 9). The resulting attenuation of the stretched chromosomes is extreme, often appearing to approach the breaking point (note especially the middle bivalent of Figure 8). A pronounced asynchrony characterizes the stretching process among the different bivalents. In its early stages, as pointed out above, there seems no correlation between position in the spindle and time and degree of stretch (Fig. 6); later stages however show the most extreme stretch in bivalents equatorially placed on the spindle, while those nearer the poles are belated in opening (Figs. 8 and 9). Eventually all the bivalents are stretched open,—but the asynchrony is so great that the stretching process actually overlaps the recontraction of the bivalents which follows it.

Due to the shortness of the pairing segments in the sex chromosomes, their kinetochores are never so close together as those of the autosomal bivalents. Furthermore, the chromosomes of the sex trivalent, unlike those of autosomal bivalents, begin to separate and have already assumed an end to end alignment (X_1–Y–X_2) before the stretching process is initiated. (This point is difficult of demonstration in Stagmomantis but is clearly evident in Paratenodera and Choeradodis.) As the spindle forms in the nuclear area the kinetochores of the sex trivalent move toward the poles. Their movement, unlike that of the bivalents, is a random one,—not determined by repulsion between homologous kinetochores. Thus one X and the Y may move toward one pole and the other X toward the opposite pole; both Xs may go toward one and the Y toward the other pole; or, one X may pass toward each pole while the Y is stretched between them (Figs. 8, 9, and 10). Occasionally a trivalent shows all three kinetochores near one pole,—but it is impossible to distinguish these positively from trivalents which have not yet oriented. Apparently the direction of kinetochore movement is toward the nearer pole, determined by the chance position of the chain of three chromosomes at the time of the formation of the spindle.

b. Re-approach of homologous kinetochores.

The extreme stretching of the meiotic chromosomes is followed by their gradual re-contraction and the re-approach of their widely separated kinetochores. This movement seems to be brought about by the resumption of the normal coiling of late prophase, previously interrupted by the stretching process. A slight but consistent decrease in spindle length during the assumption of the compact form of final metaphase (measurements in Table I) undoubtedly expedites the process. The outline of the chromosomes becomes smoother and their staining capacity greater as contraction proceeds, suggesting the deposition of matrical material during this time. This feature, together with the degree to which the bivalent has been opened, permits one to distinguish between those bivalents in process of stretching and those undergoing the ensuing contraction. The precise time at which half spindle components form between the kinetochores and the centers is uncertain, due to the difficulty of differentiating them from the mass of fine fibrils making up the continuous spindle. However, at the period of maximum stretching many bivalents show unmistakable half spindle components. The re-contraction of the stretched chromosomes, and the re-approach of the homologous kinetochores, is thus effected despite any pull or resistance that may be offered by the half spindle components.

Table I

Kinetochore movement and spindle elongation

Measurements in ocular micrometer units; each figure is the average of some ten measurements.

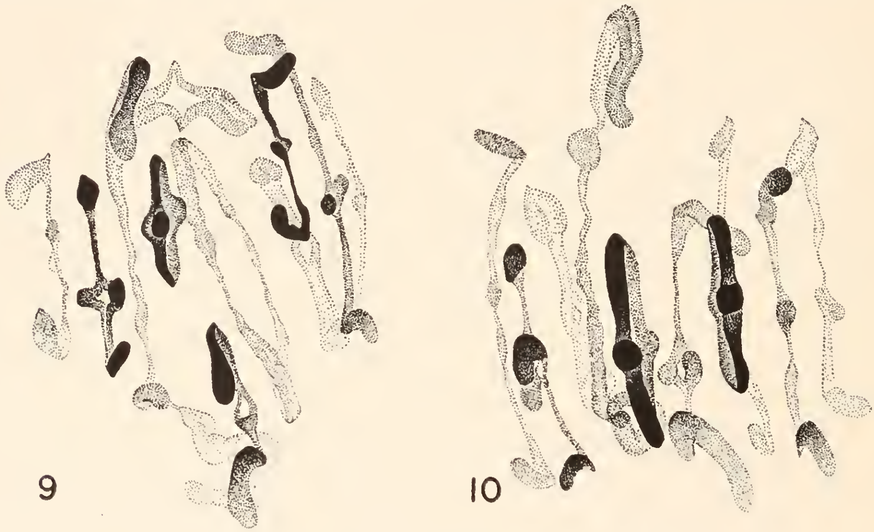
Species	Inter-center Distance				Spindle elongation during stretch	Inter-kinetochore Distance			Kinetochore separation during stretch	Kinetochore separation at metaphase
	Pre-stretch: membrane intact [1]	Early stretch: no membrane, no plate	Late stretch: plate forming	Compact metaphase: plate completed		Pre-stretch; membrane intact [1]; (av. max. sep.)	Maximum stretch; (in longest rod bivalent)	Compact metaphase; (in longest rod bivalent)		
Stagmomantis carolina, Va.	14.4	26.3	25.9	23.0	11.9	0.0	13.0	7.0	13.0	6.0
Stagmomantis carolina, B. C. Is.	19.0	24.2	24.1	22.7	5.2	2.0	10.5	7.2	8.5	3.3
Paratenodera sinensis	16.8	24.0	23.1	21.6	7.2	0.0	9.2	6.6	9.2	2.6
Liturgousa annulipes	11.5	24.5	23.9	24.0	13.0	2.1	10.6	9.2	8.5	1.3
Choeradodis rhombicollis	26.3	28.7	27.6	26.6	2.4	2.0	7.1	6.1	5.1	1.0

[1] Except in Liturgousa; here the inter-center distance recorded is the maximum attained before the orientation of the kinetochores to the centers but after the breakdown of the nuclear membrane.

The extent of these two opposite movements of the kinetochores,—first the violent movement of homologous kinetochores toward opposite poles, second their gradual re-approach to assume the position characteristic of metaphase,—is shown by measurements of the distance between the kinetochores of a given bivalent at the different stages. In the longest rod-shaped bivalent at the period of maximum stretch the distance between opposing kinetochores is 13 ocular micrometer units; at metaphase it is 7 units (each figure is the average of measurements in 10 nuclei). Since there occurs almost no separation of kinetochores prior to spindle formation in the Virginia Stagmomantis, the first figure gives the relative distance traveled by the kinetochores in their pre-metaphase poleward movement. In their re-approach the kinetochores retrace nearly half this distance. I would emphasize again the asynchrony of the different bivalents in these two movements; the stretching of laggard bivalents continues *pari passu* with the contraction of those which were first stretched open. Thus the kinetochores of one bivalent may be moving apart, toward opposite poles, while those of others are re-approaching each other.

c. Formation of metaphase plate

While the contraction of the bivalents and the re-approach of their kinetochores is under way, a new movement of the chromosomes is initiated. From a dispersed distribution through the whole spindle, the bivalents gradually shift into the equatorial region (Figs. 9 and 10). Each bivalent retains its bipolar orientation during this movement and moves as a whole toward the equator. The asynchrony noted in the two preceding movements is maintained in this also: movement toward the equator proceeds concomitantly with the re-contraction of the bivalents, and may even overlap the stretching of the most belated of them. The spacing of the chromosomes at the equator is at first wide and open—but as the maximum degree of contraction is reached they move in and form a fairly closely spaced metaphase plate. The accuracy of the



FIGURES 9 AND 10. *Stagmomantis carolina*, Virginia.

FIGURE 9. Late stretch; one bivalent still unopened; movement to equator started; X_1X_2Y in reorientation. Feulgen.

FIGURE 10. Early stage in metaphase orientation; X_1X_2Y mal-oriented. Feulgen.

seriation of stages in these movements of late prophase is attested by the fact that early anaphases always show the chromosomes in the compact form and closely spaced arrangement of the final metaphase.

Perhaps the most baffling feature of the metaphase orientation lies in the movements of the sex trivalent. We have seen that it assumes, at random, a variety of orientations during the stretching process. If the two Xs have moved to one pole and the Y to the other, the movement to the equator proceeds as in the bivalents simply by contraction of the chromosomes and a shifting of the whole configuration, with its orientation unaltered, into position in the metaphase plate. But if any other orientation be assumed during the stretch—if one X goes to each pole with the Y stretched between them, or if one X and Y pass to one pole while the other X goes to the opposite pole—a re-orientation ensues. One or more kinetochores shift their position and move through the spindle so as to bring the two Xs opposite to the Y on either side of the equator. Thus one kinetochore actually changes its orientation from one pole to the other and moves from a position close to one center to the opposite side of the equator —a maneuvre difficult indeed to visualize in terms of the mechanics of mitosis. Variation in the timing of the stretching of the trivalent and of its re-orientation, relative to the activities of the bivalents, makes it difficult to seriate its movements. But the trivalent shown in Figure 9 is probably undergoing re-orientation; the X near the upper pole is still under tension and maintains the orientation assumed in the stretch, while the lower X shows no tension and its kinetochore is in process of shifting toward the upper pole. What happens to the half spindle components during re-orientation would be of great interest, but I have not been able to follow it. Half spindle fibers are occasionally clearly visible during the stretch and are again well marked at metaphase; it is possible that they are lost and reform anew during the re-orientation.

Although the details of the process of re-orientation thus cannot be followed with certainty,—there can be no doubt that it actually takes place. Mal-orientation is encountered in some 50 per cent of the nuclei during the period of the stretch [56 cases were observed among 105 counted],—while at the final metaphase it is extremely rare [three cases in 105]. Clearly, therefore, the great majority of those trivalents mal-oriented during the stretch successfully re-orient by final metaphase.

III. *Bivalent structure and chiasmata*

Throughout pachytene the homologous chromosomes of each bivalent retain their close parallel association, with but a slight tendency to separate at the kinetochore region (Figs. 2 and 3). Diplotene and diakinetic stages as ordinarily recognized are absent: they are replaced by the forcible stretching open of the bivalents as the spindle forms, and the two kinetochores of each bivalent move toward opposite poles. As stretching proceeds it becomes evident that the kinetochore loop is not always the only locus of separation between homologues (note the two centrally placed bivalents in Figure 5). These openings, loops or half loops, alternate with persistently paired regions which resist the opening out process (Figs. 5 and 6). The paired segments may be terminal or interstitial, in one arm or in both; they vary in number from one to a maximum of three per bivalent. When first observable the openings between the paired regions appear to lie in the same plane, but as the tension in the kinetochore loop increases they may assume alternating planes (Fig. 6, middle left bivalent). The chromatids of each chromosome have not yet separated, and even the line of demarcation between chromosomes cannot be followed in the closely paired regions. It is thus impossible to determine whether or not chiasmata are present in the persistently paired regions. At the stage of maximum stretch, however, open cross formations are frequently encountered either at one end of a bivalent, giving the rod-shaped configuration, or at both with a resulting ring configuration (Fig. 8). In these bivalents it is clear that non-sister chromatids are associated distal to the opening of the cross.

No open cross configurations have been found in the sex trivalent. There is thus no evidence available as to whether or not the terminal adhesions of X_1, Y, and X_2 are of chiasmatal origin.

The final form of the bivalents at completed metaphase is fairly constant (Fig. 11). The most frequent complement comprises three rings and nine rods; rings vary in number from none to four per nucleus—rods show the corresponding range of 12 to eight. The open cross may persist to metaphase in from one to four bivalents, but the terminal connection is more commonly a swelling or lump, sometimes bipartite.

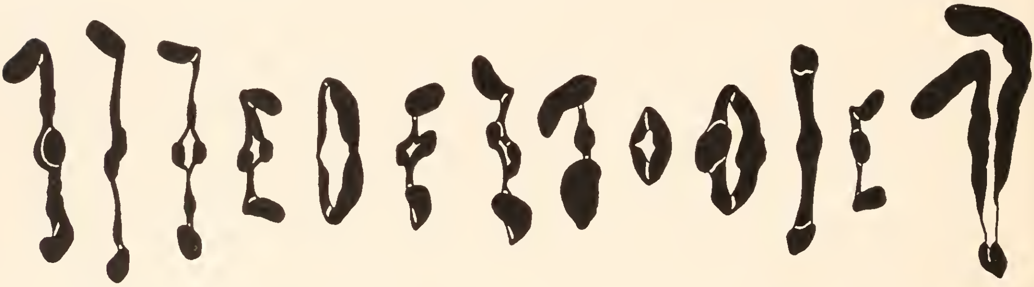
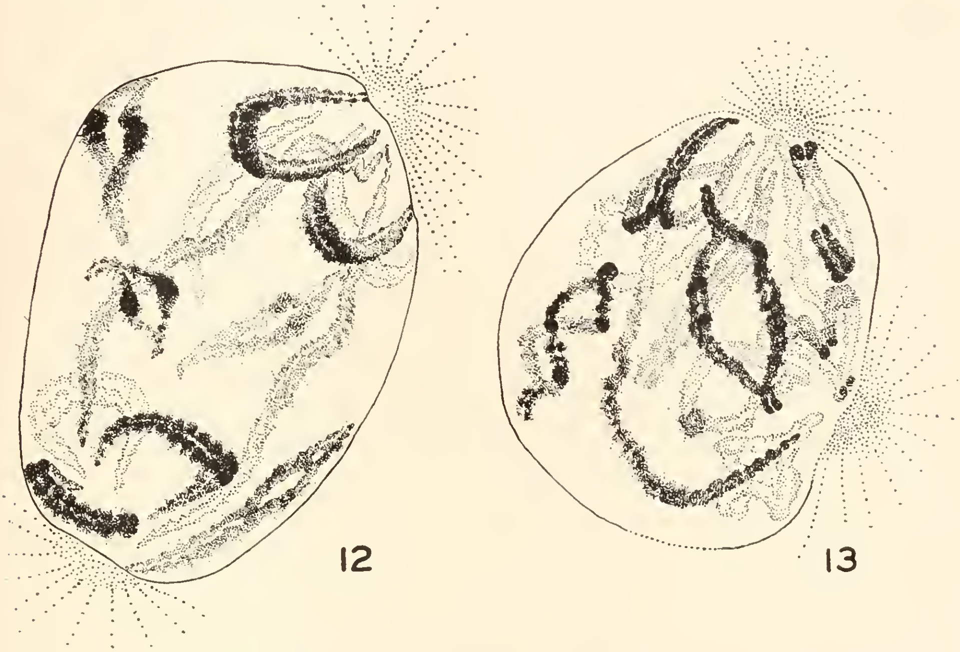


FIGURE 11. *Stagmomantis carolina*, Virginia. Bivalents and sex trivalent at metaphase. Gentian violet.

Stagmomantis carolina *from Barro Colorado Island*

Taxonomically indistinguishable, *Stagmomantis carolina* males from Barro Colorado Island and from Virginia are also identical in chromosome complement as observed in spermatogonial and meiotic metaphases. Striking and constant differences, however, characterize the meiotic prophase in the two types.

In the Barro Colorado material the pachytene polarization is initiated always at a later stage in the development of the bivalents than in the Virginian, and is of shorter duration (compare Figures 12 and 2). Moreover there is here no constant correlation between time of polarization, degree of separation of the centers, and the stage of bivalent development. Thus in Figure 13 the polarization centers are active while still relatively close together, but the bivalents are in a more advanced stage than those of Figure 12 in which the centers are already at opposite sides of the nucleus. The time of breakdown of the nuclear membrane also varies relative to the degree of separation of the centers. When the centers separate early their passage to opposite sides of the nucleus is accompanied by a marked elongation of the whole nucleus (Figure 12 is typical) along the inter-center axis. Thus when the spindle forms in the nuclear area on the collapse of the membrane, the average inter-center distance is already considerably greater than in the Virginia type. Some elongation of the spindle follows immediately on its formation but the total length achieved is somewhat less and the average amount of elongation considerably less than in the Virginia material (measurements in Table I). The maximum separation of homologous kinetochores during the stretching process is definitely less in the Barro Colorado type; this is probably dependent both on the more advanced stage of bivalent contraction and the lesser spindle elongation.



FIGURES 12 AND 13. *Stagmomantis carolina*, Barro Colorado.

FIGURE 12. Second polarization, late pachytene; early separation of homologous kinetochores. Haematoxylin.

FIGURE 13. Second polarization with centers till close together; early breakdown of membrane; advanced stage of bivalent opening. Haematoxylin.

A highly significant feature of the Barro Colorado type lies in the timing of the separation of homologous kinetochores. In the Virginia form this occurs simultaneously with the orientation of the two kinetochores of each bivalent to opposite centers—and only after membrane collapse and spindle formation. In bivalents of the Barro Colorado material, the homologous chromosomes show a marked localized separation at the region of the kinetochores while the nuclear membrane is still intact (Fig. 12). By the time the membrane gives way the separation of homologues, initially localized at the kinetochore region, has spread distally until in some cases the bivalent appears as a ring with only the terminal regions of the chromosomes still parallelly associated (Fig. 13). It is clearly evident in these stages (Figs. 12 and 13), that the plane of separation between homologous kinetochores bears no relation to the future spindle axis. This fact, together with the persistance of the nuclear membrane during the initial separation, demonstrates therefore that this first phase in the kinetochore movement is independent of centers and spindle.

With the collapse of the membrane and formation of the spindle in the nuclear area, the already widely separated kinetochores of each bivalent orient and move toward opposite poles (Fig. 14). As in the Virginia type, this first phase in the stretching process occurs while the chromosomes are scattered through the whole spindle; Figure 15 is a particularly striking example with four bivalents, all placed well above the equator, showing the movement of the kinetochores to opposite poles. In this and succeeding stages however the stretch is always most extreme in equatorially placed bivalents.

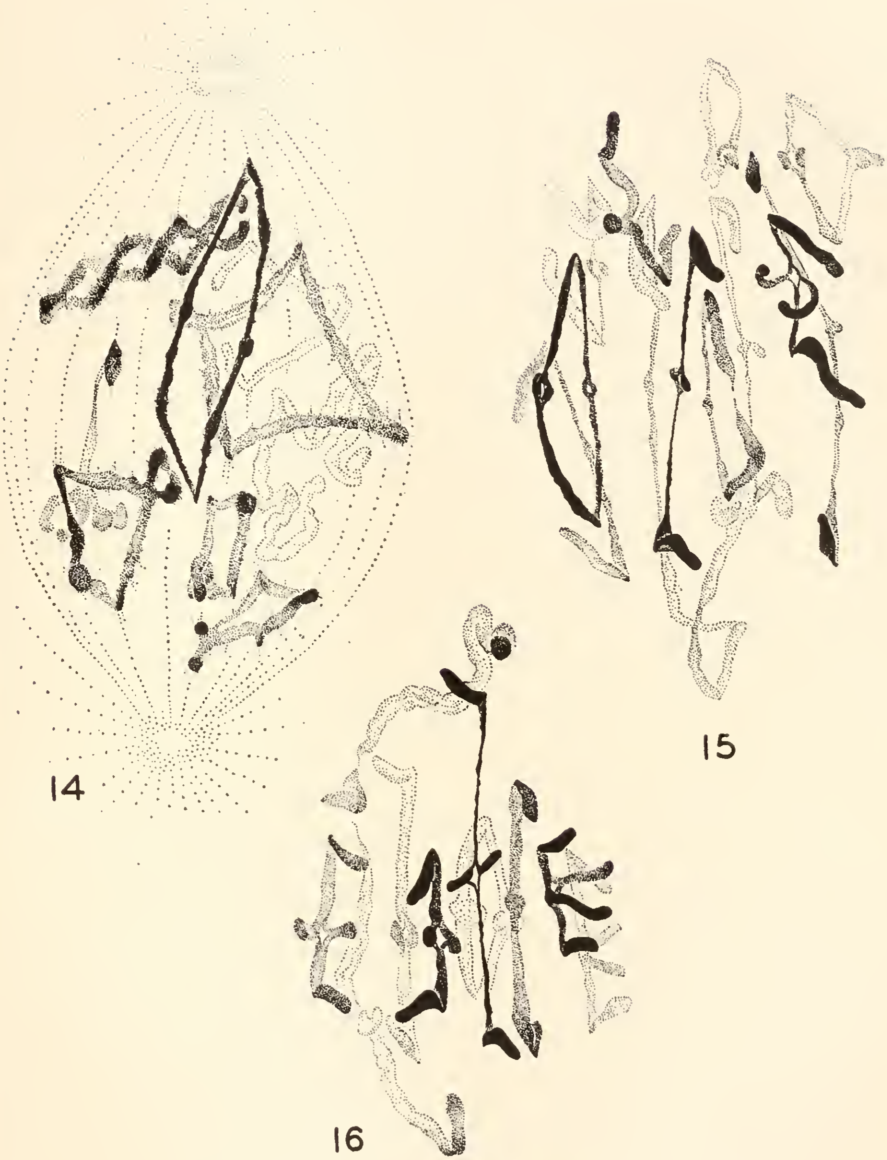
The asynchrony of the bivalents in the stretching process is equally marked in both types (compare Figures 14 to 16 with 6 to 9). In both the maximum spindle length is attained before all of the bivalents have completed the stretching process. The structure of the bivalents as disclosed during the stretch is also identical in the two types. Re-contraction of the chromosomes, re-approach of homologous kinetochores, and the movement to the equatorial plate proceed similarly. The basic difference between the two types thus lies in the timing of spindle formation relative to the stage of bivalent development. The more precocious spindle formation in the Virginia form superimposes the initial separation of homologous kinetochores and their bipolar orientation. With the delay in spindle formation in the Barro Colorado type the two processes are seen to be distinct; the initial separation of kinetochores is not determined by the centers nor the developing spindle.

Paratenodera sinensis

The chromosomes of this species have been studied and figured by King (1931) and White (1941). The diploid number of the male is 27, 12 pairs of autosomes plus X_1, X_2 and Y.

I. Second polarization

Pachytene polarization in Paratenodera presents an interesting and significant variation from the pattern observed in Stagmomantis. Usually polarization is not marked until the bivalents have condensed into short thick rods. No diplotene opening out, however, accompanies this prolonged period of contraction. The bivalents, scattered widely in the nucleus, evince some tendency toward peripheral distribution; some loose collocation of ends may persist from the leptotene zygotene bouquet, but no regular orientation is apparent. Asters form while the centers are still fairly close together. Only then do the chromosomes become definitely polarized, forming two loose aggregations close to the nuclear membrane and underlying the centers (Fig. 18). So compact are the bivalents at this stage that it is impossible to say whether their ends only or the whole mass is involved in the polarization. However the time at which the

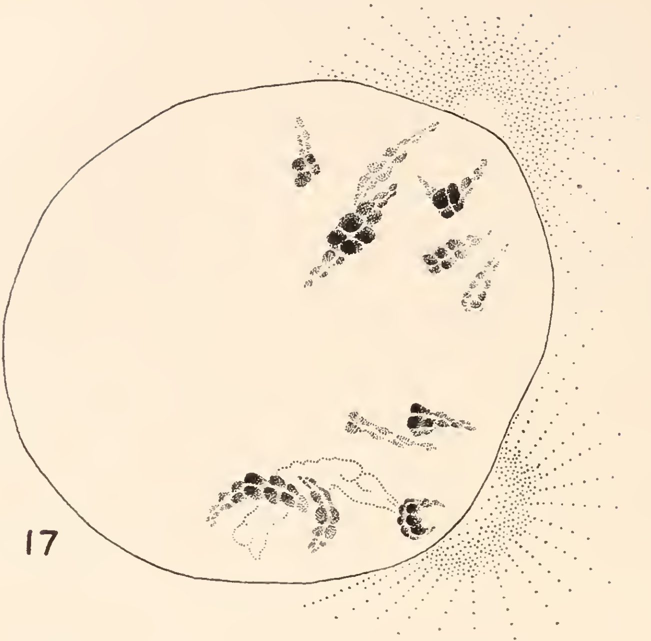


FIGURES 14 TO 16. *Stagmomantis carolina*, Barro Colorado.

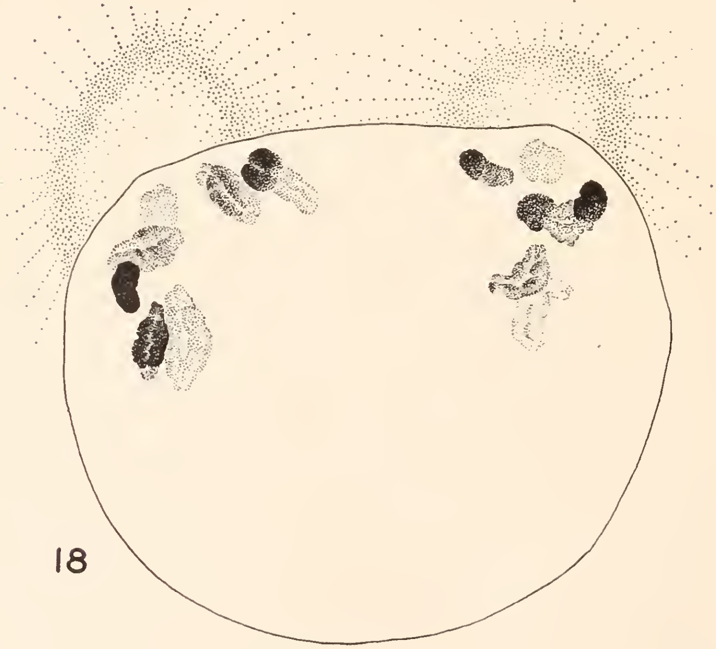
FIGURE 14. Early stretch. Haematoxylin.

FIGURE 15. Mid stretch; bivalents still scattered; early stage in open cross in one bivalent. Gentian violet.

FIGURE 16. Early stage in metaphase orientation; mal-orientation of sex trivalent. Gentian violet.



17



18

FIGURES 17 AND 18. *Paratenodera sinensis.*

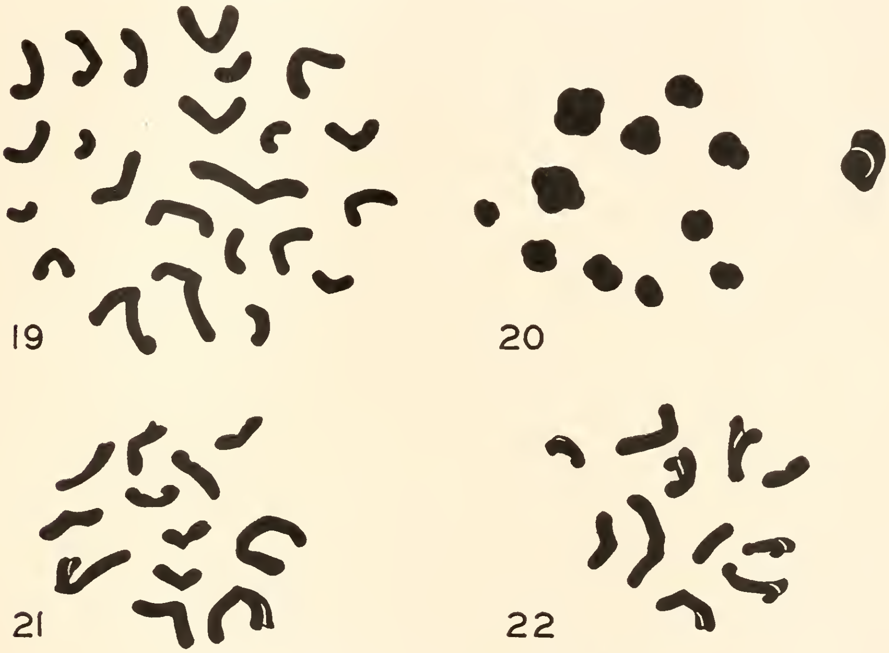
FIGURE 17. Early second polarization with chromosome ends polarized; sex trivalent in outline. Gentian violet.

FIGURE 18. Typical second polarization. Haematoxylin.

centers become active varies in relation to the stage of chromosome contraction. In a small percentage of the cells the asters form while the bivalents are still fairly long, and it is then evident that the ends of the chromosomes are specifically involved (Figure 17) just as in Stagmomantis. The Paratenodera material thus affords a series of transitional phases linking bouquet formation with the type of late prophase polarization found in Anisolabis (Schrader, 1941a) in which a specific activity of chromosome ends is not evident.

II and III. Kinetochore movements and bivalent structure

Events subsequent to the cessation of pachytene polarization follow the same general course as in Stagmomantis. Like the Virginia type of the latter, the bivalents show no marked separation of kinetochores prior to the collapse of the membrane. Formation and elongation of the spindle coincides with the initial opening out of the bivalents but the stretching process continues after spindle growth is complete. Degree of spindle elongation and intensity of stretch are intermediate between the Virginia and Barro Colorado types of Stagmomantis (measurements in Table I). Re-contraction of bivalents, re-approach of kinetochores, and movement to the equator show no significant differences from the



FIGURES 19 TO 22. *Liturgousa annulipes.*

FIGURE 19. Spermatogonial metaphase. Gentian violet.

FIGURE 20. First meiotic metaphase. Univalent X off plate at right, viewed from open end of V. Gentian violet.

FIGURE 21. Second meiotic metaphase—11 autosomes and X. Gentian violet.

FIGURE 22. Second meiotic metaphase—11 autosomes and no X. Gentian violet.

conditions in Stagmomantis. The structure of the bivalents also corresponds closely in the two genera.

Liturgousa annulipes

The chromosomes of this species have not previously been recorded. The diploid number of the male as seen in spermatogonial metaphase is 23, comprising 11 pairs of autosomes and a single X (Fig. 19). The kinetochore is approximately median in six pairs of autosomes, subterminal in five, and submedian in the X (Figs. 19 and 27). The X passes undivided to one pole at the first division and divides at the second. Second metaphase shows the expected chromosome sets of 12 and 11 (Figs. 21 and 22).

I. Second polarization

The pachytene polarization of the bivalents is very slight (Fig. 23). Many cells show none at all, and the maximum observed involves the orientation of one or both ends of at most three to five bivalents—probably those which happened to lie near the center when it became active. The time of polarization coincides, as in all the other species studied, with the first formation of astral rays. In Liturgousa, however, the latter make their appearance while the centers are still either undivided or so close together that only a single center of polarization is produced.

II. Kinetochore movements

a. Kinetochore separation, spindle formation and stretch.

As in the Barro Colorado Stagmomantis, the initial separation of homologous kinetochores is independent of division centers and spindle. This is shown, as in the former case, by the marked "repulsion" of kinetochores which occurs, in many bivalents, from mid-pachytene on (Figs. 23 and 24). Since the nuclear membrane is still intact, the future spindle axis not yet established, and the plane of kinetochore separation is random, any influence of the achromatic figure on the kinetochore movement is excluded.

The nuclear membrane breaks down while the centers are still close together (Fig. 25). The subsequent reaction of kinetochores to centers is slow in comparison with the other species, but gradually all the bivalents become oriented with one kinetochore moving toward each pole. Meanwhile the spindle forms and elongates. Due to the late separation of the centers spindle elongation after membrane collapse is greater than in any of the other species (Table I). But, as in the other forms, its maximum length is attained before the stretching of the bivalents is completed. A late stage in the stretching process, with one bivalent not yet completely opened, is shown in Figure 26.

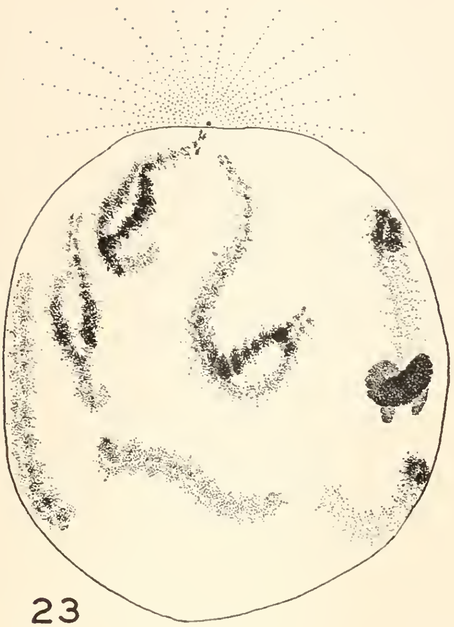
FIGURES 23 TO 26. *Liturgousa annulipes.*

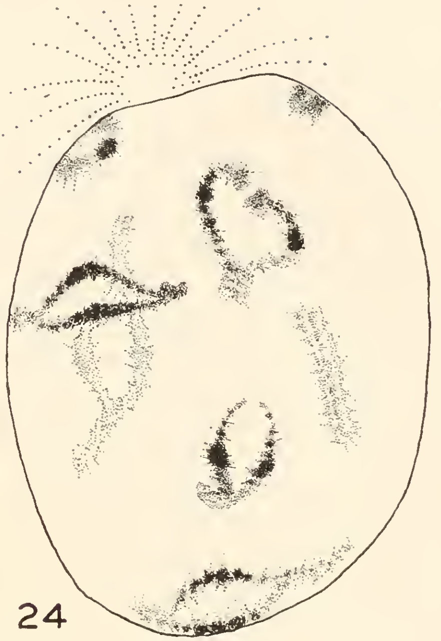
FIGURE 23. Second polarization; slight orientation of chromosome ends near center; division centers not yet separated. Haematoxylin.

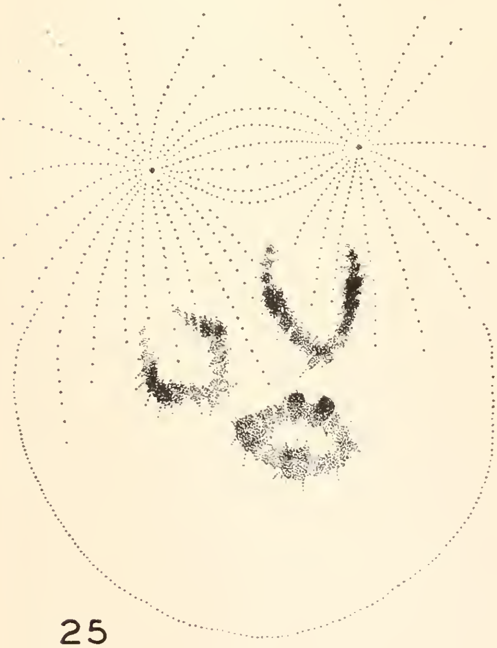
FIGURE 24. Same stage—no polarization; kinetochores separating in various planes. Haematoxylin.

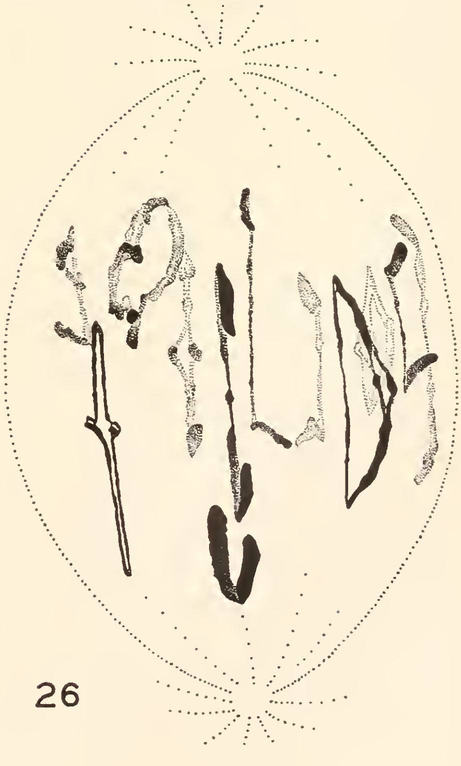
FIGURE 25. Slightly later; membrane gone; centers separating; delayed orientation of kinetochores. Haematoxylin.

FIGURE 26. Late stretch; metaphase orientation started. Gentian violet.


23


24


25


26

FIGURES 23 TO 26.

b. Re-approach of homologous kinetochores; metaphase plate formation.

The re-contraction of the bivalents, re-approach of the homologous kinetochores, and the gradual movement toward the equatorial region proceed precisely as in Stagmomantis.

III. Bivalent structure and chiasmata

In spite of the markedly early separation of the kinetochores and the consequent opening out of the bivalents before spindle formation, the chromatid structure of the bivalents is not analyzable at this stage. The separation of homologous chromosomes, at first localized in the kinetochore region, continues until a large kinetochore loop is formed, or, in some bivalents, the homologues become completely disjoined except for one short paired segment. Sister chromatids remain closely associated and cannot be traced through the persistently paired regions. That the separation of homologous chromosomes is not solely due to kinetochore movement is shown by the presence in some bivalents of a second locus of opening out in addition to the kinetochore loop or half loop. This is apparent in the separation of the ends of the horizontally placed bivalent at the mid left in Figure 24, and in all bivalents of Figure 25. After spindle formation and during the stretching process the open cross formation is encountered in several bivalents (Figures 26 and 28, a and b). The frequency of open crosses is 11.6 per cent (in 249 counted) during the stretch, and is reduced to 5.4 per cent (in 351) by final metaphase.

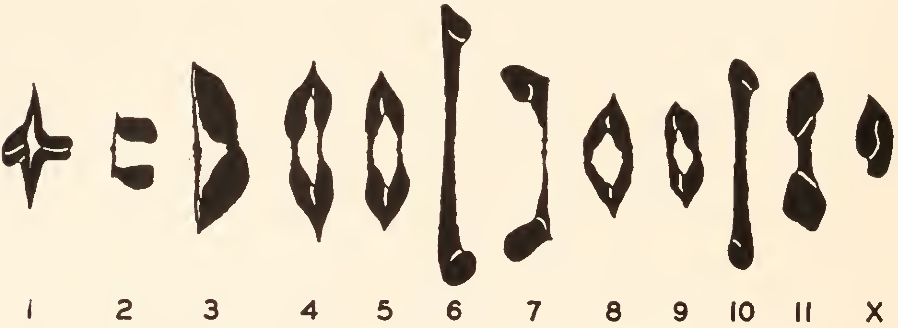


FIGURE 27. *Liturgousa annulipes.* Eleven bivalents and X at completed metaphase. Gentian violet.

The open cross configuration is usually accepted as evidence of a chiasma in process of resolution by the rotation of the arms of the bivalent. We might then assume, as White (1941) has suggested, that the terminal connections between the homologous chromosomes of the bivalents at metaphase are of chiasmatal origin. If a chiasma were present in each arm, two terminal connections would be formed—giving a ring bivalent at metaphase; if only one arm contained a chiasma, a single terminal connection and a rod bivalent would result. The behavior of two of the Liturgousa bivalents does indeed support such an argument. Easily identifiable at metaphase is the small bivalent number 2 of Figure 27 in which a terminal connection between the short arms always persists throughout the stretch, while the long arms are always free. The large bivalent number 3 of Figure 27 has arms of similar proportions, but in its case the long arms as well as the short ones are invariably connected. Moreover, in this bivalent the open cross formation may occasionally be observed in the long arms during the stretch (Fig. 28, c). Since the short arms only are subjected to stress in both bivalents, it seems reasonable to assume that the difference in behavior of the long arms is associated with the presence or absence of chiasmata. On this assumption, if no chiasma is present, the homologues separate completely and early as in the long arm of number 2, while the presence of a chiasma results in a persistent terminal connection as in the long arm of number 3.

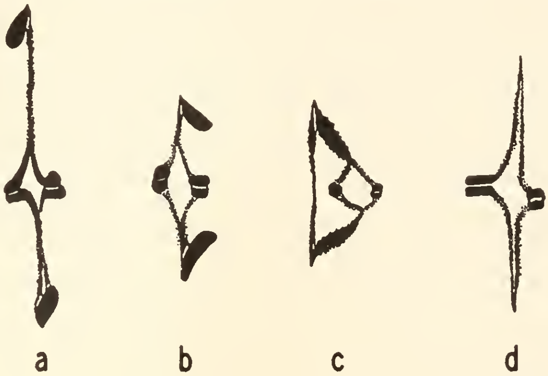
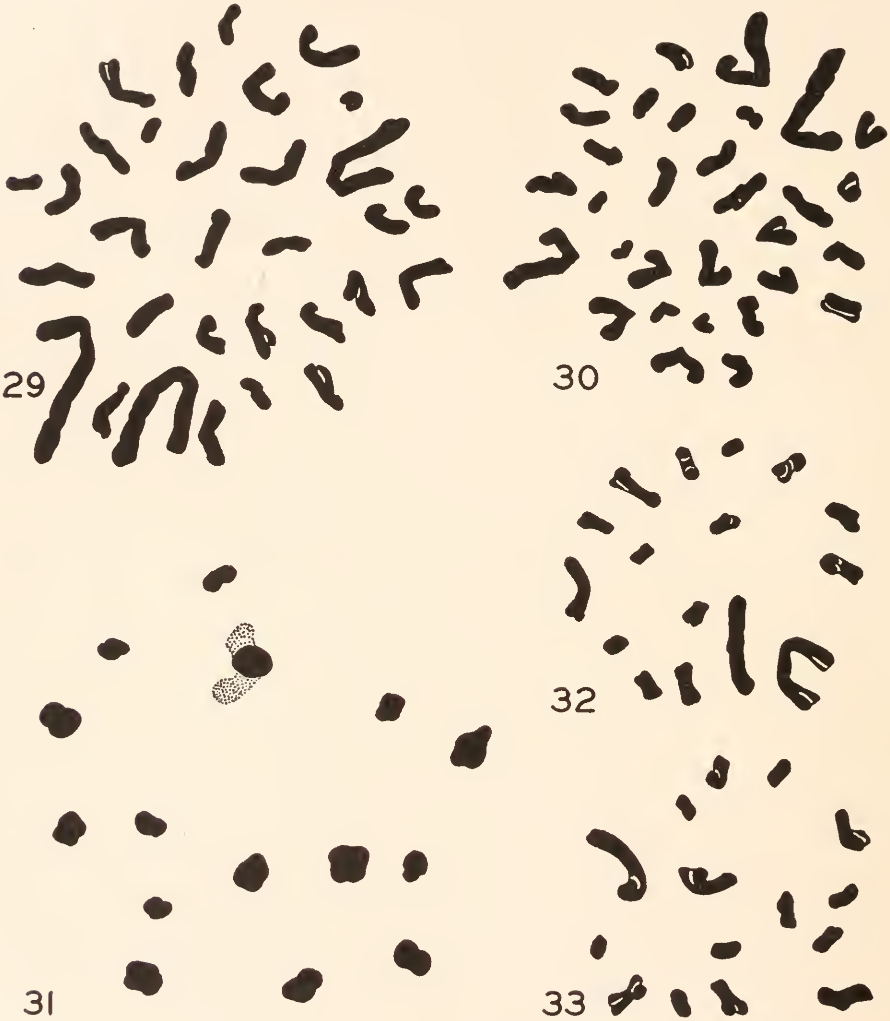


FIGURE 28. *Liturgousa annulipes.* a and b, open cross formation in rod bivalents; c, open cross in bivalent no. 3; d, bivalent no. 1 during stretch. Gentian violet.

It does not follow, however, that chiasmata are the sole and essential factor in maintaining the association of homologues. One is not justified in generalizing from the behavior of these two bivalents. Some bivalents never show the open cross or any other evidence of chiasmata; their homologues separate during the stretch with sister chromatids persistently associated; the connection between chromosome ends in these cases carries no necessary implication of previous chiasmata. Even stronger evidence is afforded by the behavior of bivalent number 1 (Figure 27). It is readily identifiable at metaphase because, alone of all the bivalents, its constituent chromosomes are in parallel association except at the kinetochore. It thus, except for a somewhat closer association of chromatids, strikingly resembles the bivalents of Callimantis (White, 1941; Hughes-Schrader, 1943) in which the absence of chiasmata in late prophase and metaphase is definitely demonstrable. But unlike Callimantis, in Liturgousa this bivalent is subjected to the same stretching process which reveals in the other bivalents of the set those open crosses and terminal connections suggestive of chiasmata. It is, therefore, evidence of a real difference in the factors determining bivalent structure to find that the homologous chromosomes of bivalent 1 separate smoothly and progressively during the stretch, disclosing no evidence of chiasmata, and making no terminal knots or adhesions. The paired region at the end of each arm decreases steadily in extent as the bivalent is stretched, but retains some parallel orientation of its chromosomes as long as it can be followed (Fig. 28, d). Re-contraction of bivalent 1 after the stretch brings the separated chromosomes back into parallel association throughout their length, except at the kinetochore. If the absence of chiasmata permits the chromosomes of the long arm of bivalent 2 to separate early and completely, why do the arms of number 1, apparently equally devoid of chiasmata, retain the parallel association of their homologues? Conversely, the pairing force operative in number 1



FIGURES 29 TO 33. *Choeradodis rhombicollis.*

FIGURE 29. Typical spermatogonial metaphase. Gentian violet and erythrosin.

FIGURE 30. Same, with maximum association of homologues. Gentian violet and erythrosin.

FIGURE 31. First meiotic metaphase; sex trivalent at upper center with Y at top focus and 2 Xs, stippled, at lower focus. Gentian violet.

FIGURE 32. Second meiotic metaphase, 14 autosomes plus 2 X. Gentian violet.

FIGURE 33. Same, with 14 autosomes plus Y. Gentian violet.

would seem to be absent in the long arm of number 2. Variation in the factors determining both the association of homologous chromosomes and the form of the metaphase configurations is thus clearly indicated for different bivalents within a single complement. Further consideration of these data is given in the discussion.

Choeradodis rhombicollis

The diploid number in the male of this species, as determined in spermatogonia, is 31—the highest number thus far found among mantids (Figs. 29 and 30). Williams' (1938) count of 27 is based on a single adult fixed without dissection, and his figures indicate that the material was inadequate to establish chromosome number and behavior.

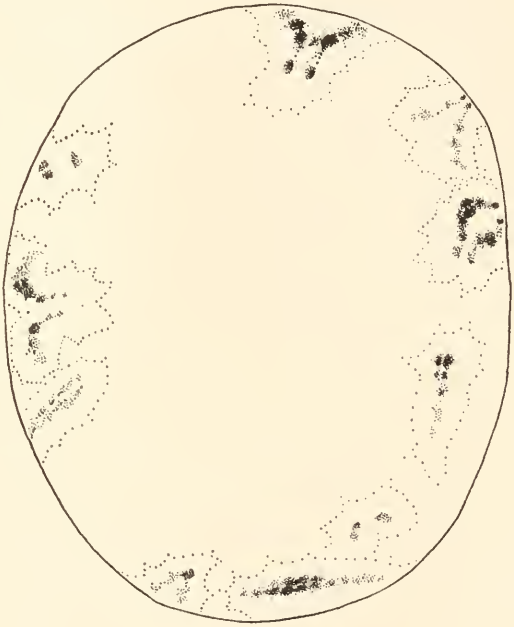


FIGURE 34. *Choeradodis rhombicollis.* Pseudo-diakinesis following diffuse late pachytene. No polarization; bivalents peripheral; erythrosin stained coagulum about chromosomes. Gentian violet and erythrosin.

The chromosome complement embraces 14 pairs of autosomes, four with median, 10 with subterminal kinetochores, and 3 sex chromosomes, X_1 X_2 and Y, with submedian kinetochores. Homologous chromosomes tend to lie near one another at spermatogonial metaphase; Figure 30 shows the maximum association observed, Figure 29 the more typical condition. The sex chromosomes are positively heteropycnotic in spermatogonial prophase and telophase, and lag in the anaphase movement.

Fourteen bivalents and a sex trivalent are formed at meiosis (Fig. 31). In general structure and behavior the trivalent corresponds with those described for other species. The proportions of the arms and the pairing relations at metaphase are shown in Figure 38. At the first meiotic division the 2 Xs pass to one pole, the Y to the other; the second metaphase accordingly shows either 15 or 16 elements (Figs. 32 and 33).

I. Second polarization

Leptotene, zygotene, and early pachytene stages correspond closely to those of the other species studied. In late pachytene however, the chromosomes become diffuse in outline, are almost unstainable in gentian violet and haematoxylin, and give but a faint Feulgen reaction. When later they again become stainable, the bivalents, already greatly shortened compared to the long threads of early pachytene, are found peripherally distributed close to the nuclear membrane (Fig. 34). Asters form about the centers, and these separate quickly to opposite sides of the nucleus, which may elongate slightly along the inter-center axis (Fig. 35); but the polarization is always slight and many do not reach the polar regions. So late is the polarization relative to the stage of contraction of the chromosomes that the latter appear to move as wholes. In a few nuclei, however, polarization occurs while the chromosomes are still relatively long, and then, as in Paratenodera, the ends alone orient to the center.

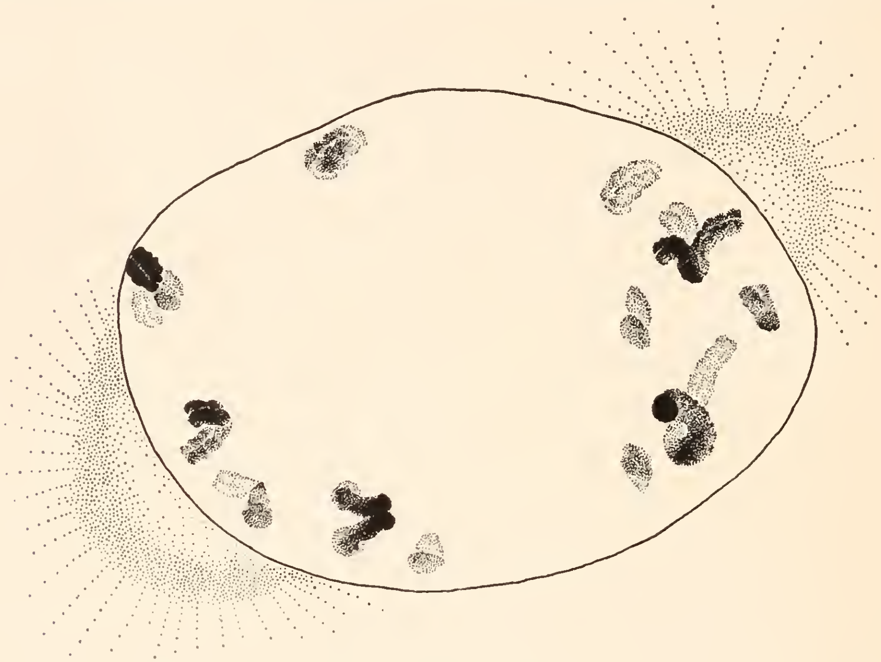
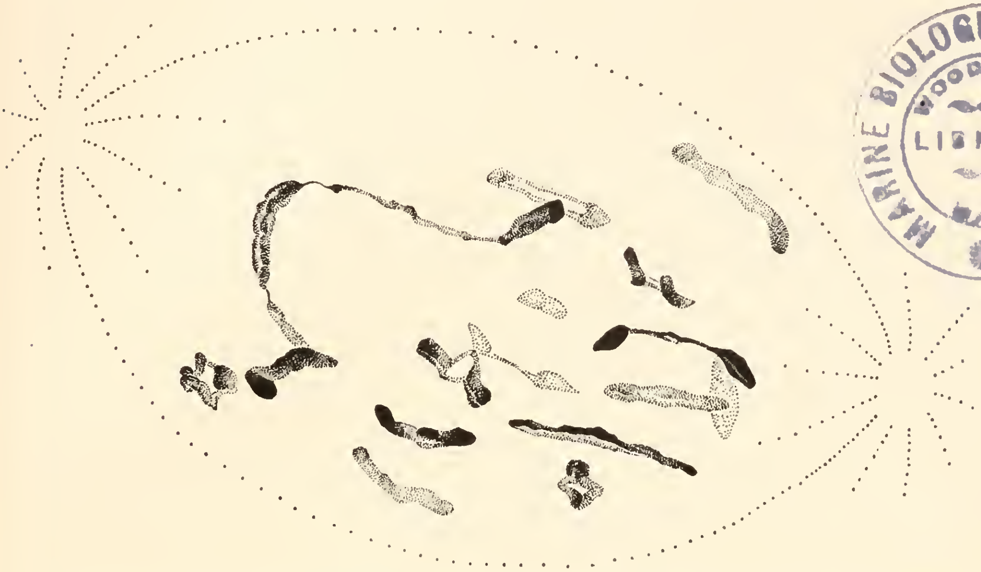


FIGURE 35. *Choeradodis rhombicollis.* Second polarization; chromosomes loosely aggregated near division centers. Haematoxylin.

II. Kinetochore movements

On the breakdown of the nuclear membrane the spindle forms in the nuclear area and quickly elongates. Due to the large size of the nucleus and the consequent wide separation of the centers, the length of the spindle is considerably greater than in any of the other species,—but the extent of its elongation is relatively slight (comparative measurements in Table I). As in the other species, spindle elongation is completed before the opening out of many of the bivalents. Indeed the orientation and movement of homologous kinetochores toward opposite poles takes place very gradually and with pronounced asynchrony in this species. Figure 36 is typical; the spindle has completed its elongation; of the bivalents some still retain the parallel association of their homologues except at the kinetochores, while others have completed the stretching process. The degree of stretching is very slight compared to the other species; the maximum separation of kinetochores observed is less than a third of the length of the spindle. The contraction of the bivalents to their final metaphase form is similarly slight. The further movements of the chromosomes to the equator and their orientation in the metaphase plate proceed as in the other species. The behavior of the sex trivalent parallels in essentials that of Stagmomantis. The early terminal alignment of the sex chromosomes, prior to the breakdown of the nuclear membrane, is especially clear in Choeradodis.



MARINE BIOLOGICAL LIBRARY WOODS HOLE MASS.

FIGURE 36. *Choeradodis rhombicollis.* Stretch: elongation of spindle completed; asynchrony of bivalents in opening out. Gentian violet.

III. Structure of bivalents

Unfortunately in Choeradodis, in which among all the species thus far studied the closest approach to a regular diplotene-diakinetic opening out occurs, the structure of the bivalents is least analysable,—due to their small size and the diffuse condition of the chromatin in the critical stages. Certain significant features can, however, be established.

Already on emergence from the confused stage of late pachytene the bivalents show a marked degree of separation between homologues (Fig. 34). This may be uniform along their entire length, or variously accentuated in different regions. If uniform, the homologues are clearly separated and indubitably devoid of chiasmata (Fig. 37, a, after Feulgen, and e, gentian violet). [Occasionally a cross section of such a bivalent shows all four chromatids equally spaced (Fig. 37, f), but such figures are rare and I have not observed chromatid separation in any others.] Prometaphase bivalents of this type probably assume a Callimantis-like form at metaphase as in bivalent 2 in Figure 38, or if more widely opened, produce simple rings such as bivalents 1 and 3 in the same figure.

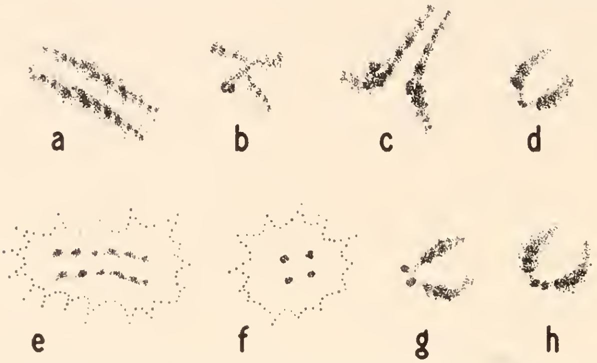


FIGURE 37. *Choeradodis rhombicollis.* Individual bivalents at pseudo-diakinesis. a–d, Feulgen; e–h, gentian violet.

In other bivalents, homologues are moderately and uniformly separated at one end, but flare widely apart at the other as though mutually repelling each other (Fig. 37, e). If at metaphase a terminal attraction operates between the paired ends when the poleward movement of the kinetochores has brought them into apposition, a rod-shaped bivalent, with no implication of previous chiasma, will result.

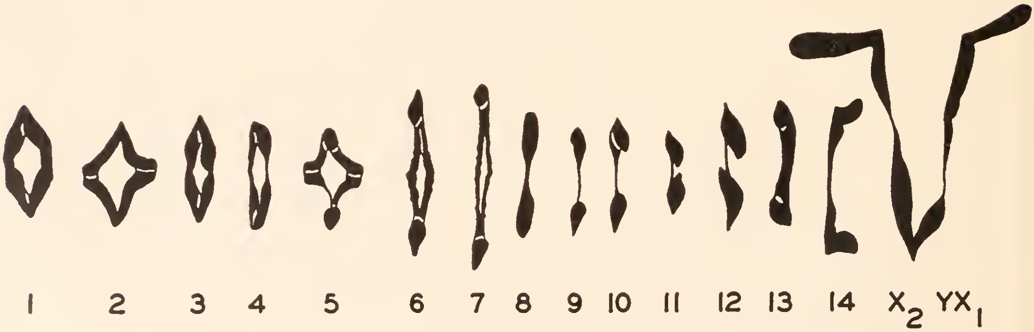
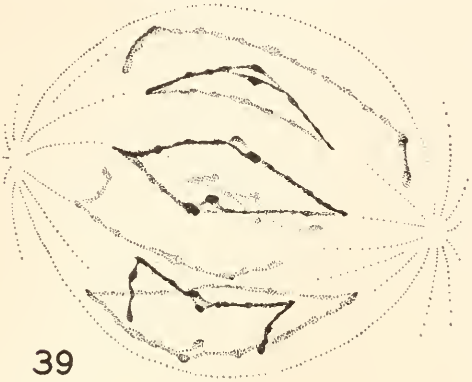
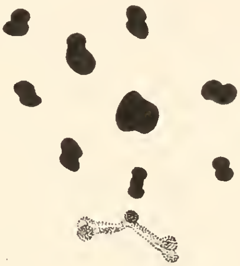
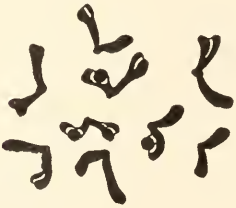


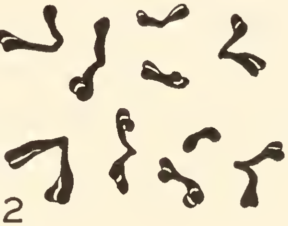
FIGURE 38. *Choeradodis rhombicollis.* Bivalents and sex trivalent at first metaphase. Gentian violet.

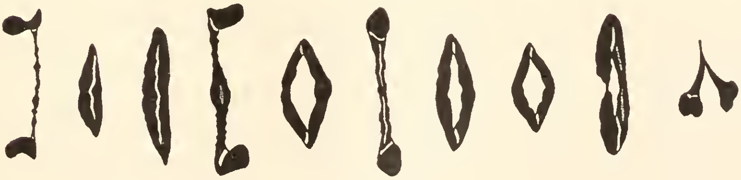
A third type of association is shown in Figure 37, b, d, g, and h. Here the persistently paired region, usually terminal or subterminal, is very short and the homologues separate widely throughout the rest of their length, with maximum separation usually at the kinetochore region. Homologous chromosomes are in contact in the paired region, but since chromatids cannot be distinguished it is impossible to tell whether or not a chiasma is present. The possibility that chiasmata are involved in these contact points is strengthened by the occasional occurrence of open cross formations in the later stages (Fig. no. 5). The frequency of the open cross is low,—slightly less than 1 per cent at metaphase.


39


40


41


42


43

FIGURES 39 TO 43. *Angela guianensis.*

FIGURE 39. Stretch stage. Haematoxylin.
FIGURE 40. First meiotic metaphase; X lies off plate at low focus. Gentian violet.
FIGURES 41 AND 42. Second meiotic metaphases. Gentian violet.
FIGURE 43. Bivalents and X at compact metaphase. Gentian violet.

Taken as a whole the Choeradodis data support the conclusion derived from the Liturgousa bivalents: the factors determining the association of homologous chromosomes in late prophase and the form of the bivalents at metaphase vary in different bivalents of a single species. Additionally Choeradodis demonstrates that in certain cases a pairing force independent of chiasmata is variously localized along the bivalent.

Angela guianensis

My material of this species is limited to a single adult male, in which the stages of spermatogenesis are incompletely represented. Its chromosomes have not previously been described. The diploid number in the male is 19, determined from meiotic metaphases (Figs. 40 to 43). Six pairs of autosomes and the univalent X have median—the others submedian kinetochores. At metaphase the bivalents assume the familiar rod or ring form; the number of rods varies from 3 to 5, of rings from 4 to 7; the most common complement being 3 rods and 6 rings (Fig. 43).

Early prophase is typical. Pachytene polarization is represented by but few nuclei but these indicate that it occurs relatively early as in the Virginia Stagmomantis. The most striking feature of the prophase is the extreme delicacy of the chromosomes at the time of stretching—a chromomeric structure is still present during the period of the stretch (Figure 39). Spindle formation seems to be more precocious than in any other species. The spindle is very compact, nearly spherical in form, and the stretched chromosomes are curved in conformity with it. During the stretching and subsequent re-contraction of the bivalents, open cross configurations are frequent,—nearly every nucleus in these stages showing two or three. These are resolved and are succeeded by terminal connections in final metaphase.

The low chromosome number, precocity of spindle formation, and frequency of open cross formation suggest that this species would reward further study.

Discussion

I. Polarization

The second polarization of chromosomes in the mantid prophase is of especial significance in the analysis of the bouquet stage—so characteristic a feature of meiosis in many animals, and to a lesser extent, plant species. The interpolation of a non-polarized stage just prior to the second bouquet demonstrates that the latter cannot be interpreted simply as a passive relic of previous telophase orientation, but involves an active orientation of chromosomes. Furthermore, the second bouquet occurs at a stage more open to analysis than is the leptotene. It is thus possible to demonstrate the participation of three structural elements of the cell in the process of polarization: (1) the orientation movements are performed by the ends of the chromosomes; (2) the division center determines the focus of aggregation; and (3) the nuclear membrane is involved in polarization.

Evidence on all these points has long been available. Recognition has been delayed due to a confusion of two distinct types of orientation: (1) the active orientation of chromosomes to membrane and center so frequently encountered in meiotic prophase, and (2) the superficially similar Rabl orientation—a passive relic of previous telophase orientation—which has been demonstrated with perhaps equal frequency in mitotic prophase. Confusion has arisen because in meiotic division the first process is sometimes superimposed upon the second. If the true leptotene bouquet is formed in cells which retain the pre-meiotic telophase orientation of their chromosomes it may well be impossible to discriminate between two distinct but simultaneously acting factors—the Rabl or relic orientation and the active orientation of bouquet formation. A third factor may further complicate polarization; it lies in the mutual attraction, at certain phases in the mitotic cycle, of heteropycnotic regions of the chromosomes. If heteropycnosis is terminally localized in the chromosomes and the attraction operates simultaneously with peripheral distribution and a polarizing activity of the center, a bouquet formation will result (as, for example in Phrynotettix (Wenrich, 1916), Stauroderus (Corey, 1933, 1938), and several species of Edessa (Schrader, 1941b)). The numerous well established cases in which bouquets form without heteropycnosis, as well as those in which heteropycnotic regions aggregate without bouquet formation, show that two separate processes are involved.

The recent revival of the idea that the bouquet stage is conditioned by previous telophasic orientation (Atwood, 1937; Hiraoka, 1941; Smith, 1942) demands reconsideration of the evidence, even at the risk of repetition. The basis for the idea lies primarily in the large body of evidence establishing the relative immobility of chromosomes during the resting stage. The aggregation of the kinetochores close to the division center in late anaphase brings the chromosome arms into a parallel or radiating arrangement. The closing in of the new nuclear membrane might then tend to bring the ends together. Granted relative immobility through the resting stage, an approximation of the bouquet arrangement would thus be already determined by the preceding telophase orientation.

The evidence against this interpretation of the bouquet has been presented by Schrader (1941b); I will summarize it here. First, telophasic reorganization will tend to bring chromosome ends together only if the kinetochore is median or nearly so. The formation of a typical leptotene bouquet in Phrynotettix (Wenrich, 1916) whose chromosomes have effectively terminal kinetochores thus shows that half of the chromosome ends have moved through the nucleus and oriented at the pole opposite that approached at telophase. Again, the hypothesis would demand a definite and fairly uniform chromosome length. In Choeradodis, with marked size differences among its chromosomes, the leptotene bouquet shows the kinetochore of the shortest element to be relatively close to the pole at which the ends are aggregated, while in only the longest chromosomes with median kinetochores do the latter retain their telophasic position. Third, typical bouquets occur in several Hemiptera (Geitler, 1937; Schrader, 1941b) although a kinetochore-center telophase aggregation cannot here—due to the diffuse nature of the kinetochore—be a causal factor (see, however, some contrary evidence in Ris, 1942). The converse of this argument is also applicable: many forms with pronounced aggregation of kinetochores at the center in telophase fail to show any bouquet stage in the succeeding meiotic prophase.

Again, we are confronted in many cases with an apparent shift in the spatial relations of center and kinetochores between telophase and meiotic prophase. Thus in Locusta (Mohr, 1916) the median kinetochores are aggregated near the center in telophase but prophase shows them at the opposite side while the chromosome ends are now grouped at the center. None of the suggestions seeking to reconcile these conditions independently of active chromosome orientation, such as a rotation of the nuclear contents through 180° (Janssens, 1924; Geitler, 1934) or a migration of the center through a similar arc (Schreiner and Schreiner, 1906; Gelei, 1921), meets the basic objection that polarization in the bouquet involves a more precise orientation and pronounced focussing of ends to a single circumscribed region than any telophasic orientation and reorganization would entail.

The formation of a typical bouquet in late prophase, following a completely non-polarized stage such as occurs in certain of the mantids, proves the meiotic polarization to be a distinct process involving forces not operative in relic orientation. The mantid evidence is valuable for its clarity rather than its novelty. Similar evidence has been available since 1921 in Gelei's careful analysis of the formation of the leptotene bouquet in Dendrocoelum oöcytes. He was able to count close to the total number of chromosome ends in the early pre-bouquet leptotene nucleus, and found them distributed, peripherally, but at random relative to the division center, throughout the nucleus.

Finally, the evidence just presented although sufficiently conclusive in itself, is secondary to the basic fact now also well established that bouquet formation involves a special activity of chromosome ends, center, and nuclear membrane.

The action of the chromosome ends in polarization is clearly evident in bouquet formation in mantids, as in many other organisms. Gelei (1921) was able to follow the movement of the knobbed ends of the Dendrocoelum chromosomes as they converged from a random distribution to aggregate in a plate-like cluster on the nuclear membrane underlying the center. The same process occurs in the formation of the second bouquet in certain mantid species. Furthermore, the variation in timing of polarization relative to degree of chromosome contraction seen in Paratenodera provides transitional stages between polarizations in which ends only are active, and those in which the whole compact body of the chromosome seems to be affected. This suggests that in the latter type, also, a special activity of ends may be involved, and adds to the growing body of evidence bespeaking special functions in these structures.

Action of the center in determining the pole of the bouquet has also long been recognized (Buchner, 1910; Ahrens, 1936, and others). It is strikingly evident in those mantid nuclei in which a single or monopolar bouquet first forms at the still undivided center and is then transformed into a double, bipolar, bouquet as the daughter centers move to opposite sides of the nucleus accompanied each by a group of chromosome ends on the inside of the membrane.

That the nuclear membrane takes a definite, if still undefined, part in polarization is becoming increasingly clear. Schrader (1941a) has shown that it plays more than a passive role in the movements of the centers as well as of the chromosomes. In the mantids the close application of terminal chromomeres to nuclear membrane, and the abrupt cessation of polarization on the collapse of the membrane, further support the thesis.

In conclusion: it appears definitely established that true bouquet formation is a special process, basically distinct from relic orientation, involving the operation of forces not active in the latter, and dependent on special activities of chromosome ends, centers, and nuclear membrane. Bouquet formation while widespread, is not, however, a universal nor essential element in meiosis. Its adaptive significance when, as is usually the case, it occurs prior to synapsis, lies in facilitating chromosome pairing, and was early recognized (v. Kemnitz, 1913; Gelei, 1921). The second bouquet stage, however, can have no such value since it occurs in post-synaptic stages. It introduces a maneuvre not ordinarily encompassed in the meiotic cycle, and one which shows the complexity of cyclical changes which may be involved in the various constituent processes of mitosis. Whatever its utility to the species, it emphasizes once again the amazing range of possible variations in the interplay of different factors in normal meiotic mechanisms.

II. Kinetochore Movements

a. Initial separation of kinetochores.

First of the several striking kinetochore movements during the meiotic prophase is the initial separation of homologous kinetochores. The first opening between the chromosomes of a bivalent is localized at the region of the kinetochores and is strongly suggestive of repulsion between these bodies. Swanson (1942) questions the efficacy of the kinetochore as a repelling body, and holds that the marked separation of kinetochores and the attenuation of the chromosome between kinetochore and nearest chiasma, usually attributed to such repulsion, is more probably due to a poleward "pull" of the spindle. Although this position appears well taken in Tradescantia, it is untenable in the mantids. Here in several species, separation of homologous chromosomes is at first limited to the kinetochore region, and occurs while the nuclear membrane is still intact. Furthermore the plane of kinetochore separation bears no relation to the position of the centers nor the future spindle axis.

Neither can we ascribe kinetochore separation to despiralization, although the fact that the latter is initiated at the kinetochore region and proceeds distally makes the assumption at first thought credible. Against it is the fact that there is no constant correlation between time of kinetochore separation and phase of spiralization cycle in the different species. Thus in the two geographic types of *Stagmomantis carolina* kinetochore separation starts at widely different points in the spiralization cycle, and in Paratenodera the bivalent has reached a contraction approximating that of metaphase before the kinetochores separate.

Again, in Callimantis (Hughes-Schrader, 1943) the differential behavior of kinetochores in quadrivalents and in bivalents shows the degree of separation to increase with the number of kinetochores present, other factors being equal. Of course, in this case, some special activity of the half spindle components, independent of the growth of the spindle as a whole, cannot be excluded, but the data seem more simply explicable on the hypothesis of kinetochore repulsion.

Thus in mantids all available evidence points to an autonomy of the kinetochores in their initial separation; the force involved is localized in or operates through the kinetochores themselves. While not establishing the nature of the force involved, the data are in harmony with the concept of a mutual repulsion between homologous kinetochores at certain stages of their cycle.

b. The stretch phenomenon.

A second and unique phase of kinetochore activity is initiated when, on the breakdown of the nuclear membrane and the formation of the spindle in the nuclear area, homologous kinetochores move suddenly toward opposite poles forcibly stretching open the bivalent between them. In bivalents with no previous repulsion of kinetochores, their separation and bipolar orientation is simultaneous; where repulsion precedes spindle formation, the kinetochores shift their position so that one points toward each pole. Obviously the force involved is operating through the kinetochores. White (1941) suggests that as the spindle forms the kinetochores become attached to it, and the spindle then elongates carrying the kinetochores toward the poles and stretching the chromosomes.

An analysis of spindle elongation and poleward movement of kinetochores is given in Table I. Spindle elongation is computed from the relative distances between the centers at the different stages; kinetochore movement similarly, in terms of inter-kinetochore distance in the longest rod shaped bivalent. Measurements are in ocular micrometer units, and each figure represents the average of some ten measurements. A correlation between spindle elongation and kinetochore movement is evident; Liturgousa and the Virginia Stagmomantis, with the greatest increase in spindle length, show the greatest kinetochore movement, while Choeradodis with the least spindle elongation has the slightest.

Despite this, spindle elongation is definitely not the only factor in the poleward movement of the kinetochores. In all but one of the five types the increase in spindle length is definitely less than the distance travelled by the kinetochores (compare columns 6 and 10 of Table I). Thus in the Virginia Stagmomantis only 5.2 units out of 8.5 may be attributed to spindle elongation; in Choeradodis only 2.4 units out of 5.1. The former case is particularly significant since chromosome length, which will be a factor in interspecific differences in degree of kinetochore separation, is the same in the two types of Stagmomantis.

Another line of evidence also demonstrates the existence of a second factor in the poleward movement of the kinetochores. It will be noted that in each case the elongation of the spindle follows quickly on its formation; thereafter spindle length remains constant or even gradually decreases up to final metaphase (Table I). The asynchrony of the bivalents in the kinetochore movement is so great that in every case the maximum spindle length is attained before all of the bivalents have opened. Since all bivalents are eventually stretched open, it is clear that in many cases the poleward movement of the kinetochores is independent of spindle elongation.

What is the nature of this second factor in the kinetochore movement? It may be simply a continuation of the mutual repulsion of homologous kinetochores already apparent in some species during the preceding stage. The orientation of the bivalent and the consequent direction of kinetochore movement would then be determined by the longitudinal structure of the spindle, permitting repulsion to act in the longitudinal axis while blocking it in any other plane. An alternative possibility exists; an attraction between center and kinetochore may come into play on the breakdown of the membrane and the formation of the spindle. Such attraction is indeed suggested by the random orientation of the kinetochores in the sex trivalent which move toward the nearer pole irrespective of their homology. The terminal alignment of the sex chromosome, completed before the poleward movement of the kinetochores begins, may result in too great a separation of their kinetochores for repulsion to be effective and thus allow the attraction between center and kinetochore to be visibly expressed.

It must not be forgotten, moreover, that half spindle components form between kinetochores and center at the time these movements are taking place. Some activity on their part, independent of the elongation of the spindle as a whole, cannot be excluded. Thus while the nature of the forces involved cannot yet be determined, the data do demonstrate that the poleward movement of kinetochores and resultant stretching of the chromosomes are due to more than one agency, and involve in addition to spindle elongation, other factors which may include repulsion of homologous kinetochores, kinetochore-center attraction, and possibly some activity of half spindle components.

c. Reapproach of homologous kinetochores.

On the completion of the poleward movement of the kinetochores, a precisely opposite action ensues. Homologous kinetochores re-approach each other and in so doing move away from the poles. The extent of this movements varies in the different species (last column of Table I). The causal factor seems to be the resumption of the normal coiling process, interrupted and partially undone by the preceding stretching.

d. Metaphase orientation.

The gradual movement of the chromosomes toward the equator of the spindle follows—and largely overlaps in time—the re-approach of the homologous kinetochores. It may even overlap the preceding stage of poleward kinetochore movement. It follows from this asynchrony that some kinetochores are still moving toward the poles while others are moving away from them, and that movement of the whole bivalent toward the equator may be superimposed on one or both of the others. The hypothesis that equatorial orientation is caused by repulsion between kinetochore and center is thus either untenable or requires the subsidiary assumption of a reversal of charge in the kinetochores—a reversal occurring, moreover, at different times in different bivalents. Again, since the spindle is completed long before movement of chromosomes to the equator begins, the latter cannot be attributed to ingrowth of spindle fibers from the poles pushing the scattered chromosomes to the equator. Finally, since both the re-approach of homologous kinetochores and the movement to the equator occur subsequent to the formation of half spindle components, a considerable elasticity in the action of these elements is indicated.

During the movement to the equator the bivalents shift position as wholes retaining their bipolar orientation. It is thus impossible to tell whether the movement is dependent on kinetochore action, as is well established in other forms. In the sex trivalent, however, the kinetochores definitely take the lead in metaphase orientation. In the re-orientation of its three elements the kinetochores alone are active while the arms appear relaxed. As to what force or forces underlie the movement to the equator and the co-orientation of homologous kinetochores, the present data give no clue.

III. Chiasmata and bivalent structure

Cases already on record demonstrate that chaismata are not the sole, nor an essential, factor in maintaining the meiotic association of homologous chromosomes in late prophase and metaphase (references in Hughes-Schrader, 1943).

Bivalents clearly devoid of chiasmata may retain the parallel association of their chromatids. Terminal connections between homologues at metaphase may be formed in complete independence of previous chiasma formation. We may take it as established that different species vary in the factors involved in the later stages of the meiotic association of chromosomes.

The present study shows further that (1) such variation exists even among closely related species, and (2) that bivalents within a single species may similarly vary in the factors determining the association of their homologues. White (1941) was unwilling on the evidence then available to admit such variation in a basic meiotic mechanism in so closely related a group of species as the mantids. Confronted on the one hand with the absence of chiasmata in the bivalents of Callimantis, and on the other with contrary although indirect evidence in all other species studied, he holds that the differences are superficial only. This implies either that the indirect evidence for chiasmata in the majority of species will prove unfounded or, as White further suggested, that chiasmata may be present in the Callimantis bivalent, but, in the absence of the stretch phenomenon in that species, are not disclosed due to the close parallel association of homologous chromosomes up to anaphase.

As to the first alternative, the present study offers strong presumptive evidence for the occurrence of chiasmata in certain bivalents of some species. The evidence lies in the relatively high frequency of open cross formations in opening bivalents, in which the association of non-sister chromatids distal to the opening of the cross may be clearly demonstrated. That such configurations necessarily imply previous chiasmata cannot be taken as completely proved (contrary evidence in the somatic chiasmata of Drosophila ganglion cells, Kaufmann, 1934), but the weight of evidence from many sources (heteromorphic bivalents, interlocking, etc.) is certainly in favor of this interpretation.

The second alternative is definitely untenable in the light of the evidence now available. Re-investigation of Callimantis (Hughes-Schrader, 1943) has confirmed White's (1938) earlier conclusion that chiasmata are absent. Neither in the parallel association of four chromatids in the prometaphase and metaphase bivalent nor in its anaphasic disjunction are chiasmata involved. The present study offers additional evidence that the Callimantis type of bivalent on the one hand, and the rod and ring types of other mantids on the other, present real differences in structure and in the forces underlying the association of the homologues. The differences in the metaphase form of bivalents are not ascribable simply to presence or absence of the stretch phenomenon. Thus we have seen in Liturgousa one bivalent of the Callimantis type, subjected to the same stretching that results in rod and ring formation by the other bivalents, open out without terminal connections and on re-contraction resume the parallel association of its homologues. Conversely, in Choeradodis, where the stretching of the bivalents on the spindle is almost nil, rod and ring types are nevertheless produced. In view of these lines of evidence the conclusion is justified that even closely allied species vary in the factors underlying the late meiotic association of homologous chromosomes.

Within the single species, also, the presence of more than one factor is demonstrable. Thus in the case of Liturgousa cited above, factors other than chiasmata determine the association in one bivalent, while others as consistently disclose the open cross with its implication of previous chiasmata. In Choeradodis, also, diversity of factors is evident.

Three separable factors in the late meiotic association of homologous chromosomes can be distinguished. They may operate separately or in various combinations in bivalents of different species and in different bivalents of one species. The first of these factors—the pairing force independent of chiasmata which we must assume determines the persistent parallel association of chromatids in the Callimantis type of bivalent—I shall term, for brevity in discussion, lateral attraction. The second, expressed in the formation of terminal connections between separating homologues, is recognized as terminal attraction. The term attraction is here used in a purely descriptive sense, without implication of the nature of the force involved. The third factor is the action of chiasmata. Let us consider these factors, as expressed in the structure of the mantid bivalents, separately.

Lateral attraction is demonstrated most clearly in the Callimantis bivalents where the four distinctly separated chromatids of each arm maintain their parallel association, without chiasmata, until anaphase. It is similarly expressed in the single Callimantis-like bivalent of Liturgousa, and in several of the Choeradodis bivalents whose homologous chromosomes are clearly separated but parallelly associated until stretched apart on the spindle. The data of the present study further demonstrate that lateral attraction may be variously localized in the individual bivalent. This is seen in those bivalents of Choeradodis whose homologous chromosomes show no contact during the pseudo-diakinetic period and where chiasmata are thus excluded as a factor in the association. Of these bivalents some show the chromosomes parallelly paired and equidistant along their whole length. In others the pairing segment is reduced to a short region, variously localized in different bivalents, with the homologues flaring widely apart elsewhere. A similar differential localization of lateral attraction is apparent in the diakinetic bivalents of the egg of the grass mite, *Pediculopsis graminum* (Cooper, 1939).

Terminal attraction is expressed in the resistance of apposed chromosome ends to the forces of anaphasic separation in the bivalents of Callimantis. In this case no fusion or physical connection of any kind between the chromatid ends is apparent. In the other mantid species the operation of terminal attraction is accompanied by the formation of persistent terminal connections between the ends of homologous chromosomes during the stretching of the bivalents on the spindle. So extreme is the tension produced by the movement to opposite poles of homologous kinetochores that the chromosomes are often attenuated to thin threads, and yet the terminal connections persist. In these cases, unlike Callimantis, a real fusion of certain constituents of the chromosome is suggested.

Terminal attraction may operate quite independently of chiasmata, as is shown in Callimantis and in other cases on record, perhaps most strikingly in Rhytidolomia (Schrader, 1940). This independence is also demonstrable in Choeradodis. Here several bivalents are clearly devoid of chiasmata during the pseudo-diakinetic period. Yet in the majority of nuclei at final metaphase all bivalents are of either the rod or ring type, with one or two terminal connections respectively. Obviously in certain bivalents terminal connections have formed independently of the terminalization of chiasmata.

Chiasmata are absent in late prophase and metaphase in many mantid bivalents and even when present appear to play but a subsidiary role in meiotic association. Since "repulsion" between pairs of chromatids, so diagnostic a feature of diplotene and diakinesis in most forms, is not obligatory in mantids, chiasmata when present will not be expected greatly to modify bivalent form in prophase. Nor is the form of the metaphase bivalent dependent on chiasmata to the extent which is usually assumed. We have seen that terminal connections between homologous chromosomes cannot be interpreted as invariably the sequelae of chiasma terminalization. They may also result, as is the case in certain Choeradodis bivalents, simply from terminal attraction between those ends which were held together earlier by lateral attraction and were finally brought into contact by the stretching of the bivalent. When chiasmata are present, their terminalization may well have a similar effect; it will bring chromatid ends into contact and thus possibly facilitate the operation of terminal attraction and the formation of terminal connections.

The nature of the forces involved in lateral and terminal attraction is obscure. The formation of a non-staining pellicle common to both homologues has been suggested, but no positive evidence of its existence is available. The intense staining of terminal connections in many mantid bivalents suggests a fusion of some chromosome constituent. But it must be remembered that in certain cases of terminal attraction in other forms (e.g. Rhytidolomia, Schrader, 1940) the chromosome ends move toward each other over a considerable distance and any fusion that may occur is thus secondary to the initial attraction. The same holds true for lateral attraction in the secondary metaphase pairing in spermatocytes of Lepidosiren (Agar, 1911), and in the re-association of previously separated chromatids in the second meiotic division of certain coccids (Hughes-Schrader, 1931).

Somatic pairing is similar in certain respects to the lateral attraction of meiosis. In this connection it is interesting that White (1938) finds no somatic pairing in Callimantis in which lateral attraction is so strongly expressed. Nor have I any convincing evidence of its occurrence in the species here reported, with the possible exception of Choeradodis spermatogonia, and even here the association of homologues is neither close nor constant. Oguma (1921) reports a similarly indefinite association in the spermatogonia of *Tenodera aridifolia*. Clearly there is no obligatory relation between somatic pairing and the lateral attraction of meiosis.

Summary

1. *Bouquet formation.* An analysis of the second polarization or bouquet stage in the meiotic prophase of the males of several species of mantids shows bouquet formation to be a special process, basically distinct from the relic or Rabl orientation, and involving special activities of chromosome ends, division centers, and nuclear membrane.

2. *Kinetochore movements.* (a) The initial separation or "repulsion" of the homologous kinetochores in the meiotic bivalent is shown in several species to be independent of division centers and spindle and appears to be autonomous to the kinetochores. (b) The pre-metaphase poleward movement of kinetochores and consequent stretching of the meiotic chromosomes are in part due to the

elongation of the spindle, and in part to another factor or factors which may include kinetochore repulsion, kinetochore-center attraction, and a special activity of the half spindle components. (c) Resumption of coiling after the pre-metaphase stretch results in the re-approach of the homologous kinetochores and their movement away from the poles. (d) Movement of chromosomes to the equator regularly overlaps the movement (c) above, and may overlap (b), thus excluding any hypothesis of metaphase plate formation in which the chromosomes are regarded as passive.

3. *Chiasmata and bivalent structure.* Three separable factors in the late meiotic association of homologous chromosomes can be distinguished: (a) lateral attraction, which is independent of chiasmata and is variously localized in different bivalents; (b) terminal attraction which operates in some bivalents quite independently of chiasmata, and in others follows chiasma terminalization; and (c) the action of chiasmata. Absent in late prophase and metaphase in certain bivalents, the presence of chiasmata is inferred in others from the frequency of open cross configurations. These three factors may act more or less separately, and in various combinations, even in closely allied species, and in different bivalents of a single species.

4. Males of *Stagmomantis carolina* from Virginia and from Barro Colorado Island, C. Z., identical taxonomically and in chromosome complement, differ in the time of spindle formation relative to the stage of bivalent development in the meiotic prophase.

LITERATURE CITED

AGAR, W. E., 1911. The spermatogenesis of Lepidosiren paradoxa. *Quart. Jour. Mic. Sci.*, **57**: 1–44.

AHRENS, W., 1936. Das dynamische Verhalten der Chromatinschleifen im Stadium des Buketts und das Reduktionsproblem. *Zool. Anz.*, **116**: 49–62.

ATWOOD, S., 1937. The nature of the last premeiotic mitosis and its relation to meiosis in Gaillardia. *La Cellule*, **46**: 391–409.

BUCHNER, P., 1910. Von den Beziehungen zwischen Centriol und Bukettstadium. *Arch. Zellforsch.*, **5**: 215–228.

COOPER, K. W., 1939. The nuclear cytology of the grass mite, Pediculopsis graminum (Reut.), with special reference to karyomerokinesis. *Chromosoma*, **1**: 51–103.

COREY, H. I., 1933. Chromosome studies in Stauroderus (an orthopteran). *Jour. Morph.*, **55**: 313–349.

COREY, H. I., 1938. Heteropycnotic elements of orthopteran chromosomes. *Arch. Biol.*, **49**: 159–172.

ERAZI, A. R. R., 1940. Contribution à l'étude chromosomique des Mantidae européennes. *Rev. Fac. Sci. Univ. Istanbul*, **5**: 1–24.

GELEI, J., 1921. Weitere Studien über die Oogenese des Dendrocoelum lacteum. II Die Längskonjugation der Chromosomen. *Arch. Zellforsch.*, **16**: 88–169.

GEITLER, L., 1934. Grundriss der Cytologie. Gebr. Borntraeger, Berlin.

GEITLER, L., 1937. Die Analyse des Kernbaus und der Kernteilung der Wasserläufer Gerris lateralis und Gerris lacustris (Hemiptera Heteroptera) und die Somadifferenzierung. *Ztschr. f. Zellforsch.*, **26**: 642–672.

GIARDINA, A., 1897. Primi stadi embrionali della Mantis religiosa. Nota preventiva. *Mon. Zool. Ital.*, **8**: 275–280.

HIRAOKA, T., 1941. Studies of mitosis and meiosis in comparison. IV. A contribution to the study of the origin of the "bouquet" and its formation. *Cytologia*, **11**: 483–492.

HUGHES-SCHRADER, S., 1931. A study of the chromosome cycle and the meiotic division figure in Llaveia bouvari—a primitive coccid. *Ztschr. f. Zellforsch.*, **13**: 742–769.

HUGHES-SCHRADER, S., 1943. Meiosis without chiasmata,—in diploid and tetraploid spermatocytes of the mantid Callimantis antillarum Saussure. *Jour. Morph.* **73**: 111–141.

Janssens, F. A., 1924. La chiasmatypie dans les insectes. *La Cellule*, **34**: 134–359.

Kaufmann, B. P., 1934. Somatic mitoses in Drosophila melanogaster. *Jour. Morph.*, **56**: 125–155.

von Kemnitz, G. A., 1913. Eibildung, Eireifung, Samenreifung, und Befruchtung von Brachycoelium salamandrae (Brachycoelium crassicolli (Rud.)). *Arch. f. Zellforsch.*, **10**: 470–506.

King, R. L., 1931. Chromosomes of three species of Mantidae. *Jour. Morph. and Physiol.*, **52**: 525–533.

Mohr, O. L., 1916. Studien über die Chromatinreifung der männlichen Geschlechtszellen bei Locusta viridissima. *Arch. Biol.*, **29**: 579–752.

Oguma, K., 1921. The idiochromosomes of the mantis. *Jour. Coll. Agric.*, Hokkaido Imp. Univ., **10**: 1–27.

Ris, H., 1942. A cytological and experimental analysis of the meiotic behavior of the univalent X chromosome in the bearberry aphid Tamalia (= Phyllaphis) coweni (Ckll.). *Jour. Exp. Zool.*, **90**: 267–330.

Schrader, F., 1940. The formation of tetrads and the meiotic mitoses in the male of Rhytidolomia senilis Say (Hemiptera Heteroptera). *Jour. Morph.*, **67**: 123–141.

Schrader, F., 1941a. The spermatogenesis of the earwig Anisolabis maritima Bon. with reference to the mechanism of chromosomal movement. *Jour. Morph.*, **68**: 123–147.

Schrader, F., 1941b. Heteropycnosis and non-homologous association of chromosomes in Edessa irrorata (Hemiptera Heteroptera). *Jour. Morph.*, **69**: 587–607.

Schreiner, A., and K. E. Schreiner, 1906. Neue Studien über die Chromatinreifung der Geschlechtszellen. I. Die Reifung der männlichen Geschlechtszellen von Tomopteris onisciformis Eschscholtz. *Arch. Biol.*, **22**: 183–314.

Smith, S. G., 1942. Polarization and progression in pairing. II. Premeiotic orientation and the initiation of pairing. *Canad. Jour. Res.*, **20**: 221–229.

Swanson, C. P., 1942. Some considerations on the phenomenon of chiasma terminalization. *Amer. Nat.*, **76**: 593–610.

Wenrich, D. H., 1916. The spermatogenesis of Phrynotettix magnus with special reference to synapsis and the individuality of the chromosomes. *Bull. Mus. Comp. Zool., Harvard Coll.*, **60**: 57–134.

White, M. J. D., 1938. A new and anomalous type of meiosis in a mantid, Callimantis antillarum Saussure. *Proc. Roy. Soc.*, **125**: 516–523.

White, M. J. D., 1941. The evolution of the sex chromosomes. I. The X O and X_1X_2Y mechanisms in praying mantids. *Jour. Gen.*, **42**: 143–172.

Williams, E. C., 1938. Spermatogenesis of a mantid, Choeradodis rhombicollis (Latreille). *Trans. Am. Mic. Soc.*, **57**: 387–394.

INDEX

ACETYLCHOLINE, action of, on isolated heart of Venus mercenaria, 79.
Amblystoma, neurulation in mechanically and chemically inhibited, 103.
Anaphase movement, a quantitative study of, in the aphid Tamalia, 164.
ANDERSON, THOMAS F. *See* Harvey and Anderson, 151.
Annual report of the Marine Biological Laboratory, 1.
Aphid, production of types of, and germarial differences, 52.
Aphid, a quantitative study of the anaphase movement in, 164.
Arbacia punctulata, egg, rate of breaking and size of halves of, when centrifuged in hypo- and hypertonic sea water, 141.
Arbacia punctulata, radiosensitivity of eggs of, in various salt solutions, 193.
Arbacia punctulata, spermatozoon and fertilization membrane of, as shown by the electron microscope, 151.

BODINE, JOSEPH HALL, AND THEODORE NEWTON TAHMISIAN. The development of an enzyme (tyrosinase) in the parthenogenetic egg of the grasshopper, Melanoplus differentialis, 157.
BUMPUS, DEAN F. *See* Clarke, Pierce and Bumpus, 201.
BURT, AGNES SANXAY. Neurulation in mechanically and chemically inhibited Amblystoma, 103.

CHAETOPHORACEAE, new records of, for North America, 244.
Chaetosphaeridiaceae, new records of, for North America, 244.
CLARKE, GEORGE L., E. LOWE PIERCE, AND DEAN F. BUMPUS. The distribution and reproduction of Sagitta elegans on Georges Bank in relation to hydrographical conditions, 201.
Crustacea, histogenesis and cyclic phenomena of sinus gland and x-organ in, 87.
Cytoplasmic granules, the osmotic properties of, in the sea urchin egg, 179.

DAPHNIA, analysis of population develop- in, at different temperatures, 116.
DIPPELL, RUTH V. *See* Sonneborn and Dippell, 36.
Distribution, of Sagitta elegans, on Georges Bank, in relation to hydrographical conditions, 201.

EGG, Arbacia, rate of breaking and size of halves of, when centrifuged in hypo- and hypertonic sea water, 141.
Egg, parthenogenetic, development of tyrosinase in, of Melanoplus differentialis, 157.
Egg, sea urchin, the osmotic properties of cytoplasmic granules in, 179.
ENGLE, JAMES B. *See* Loosanoff and Engle, 69.
Enzyme (tyrosinase), development of, in the parthenogenetic egg of the grasshopper, Melanoplus differentialis, 157.

FERTILIZATION membrane, of Arbacia punctulata, as shown by the electron microscope, 151.

GEORGES Bank, distribution and reproduction of Sagitta elegans on, in relation to hydrographical conditions, 201.
Grasshopper, development of tyrosinase in the parthenogenetic egg of, 157.

HABROBRACON, intersexuality and intersexual females in, 238.
HARRIS, DANIEL L. The osmotic properties of cytoplasmic granules of the sea urchin egg, 179.
HARVEY, ETHEL BROWNE. Rate of breaking and size of the "halves" of the Arbacia punctulata egg when centrifuged in hypo- and hypertonic sea water, 141.
HARVEY, ETHEL BROWNE, AND THOMAS F. ANDERSON. The spermatozoon and fertilization membrane of Arbacia punctulata as shown by the electron microscope, 151.
Heart, action of acetylcholine on isolated, of Venus mercenaria, 79.
Hemolysis, osmotic, species differences in rates of, within the genus Peromyscus, 52.
Histogenesis, and cyclic phenomena of the sinus gland and x-organ in crustacea, 87.
HOVANITZ, WILLIAM. Hybridization and seasonal segregation in two races of a butterfly occurring together in two localities, 44.

HUGHES-SCHRADER, SALLY. Polarization, kinetochore movements, and bivalent structure in the meiosis of male mantids, 265.

Hybridization, and seasonal segregation, in two races of a butterfly, 44.

INHIBITION, mechanical and chemical, in Amblystoma, 103.

Intersexuality, and intersexual females in Habrobracon, 238.

KINETOCHORE movements, and bivalent structure in the meiosis of male mantids, 265.

LAWSON, CHESTER A. Germarial differences and the production of aphid types, 60.

LEVINE, HARRY P. Species differences in rates of osmotic hemolysis within the genus Peromyscus, 52.

Life history, of the digenetic trematode, Zoögonoides laevis Linton, 227.

LOOSANOFF, VICTOR L., AND JAMES B. ENGLE. Polydora in oysters suspended in water, 69.

MANTIDS, male, polarization, kinetochore movements, and bivalent structure, in the meiosis of, 265.

MARINE BIOLOGICAL LABORATORY, annual report of, 1.

Mating types, in variety 4, Paramecium aurelia, 36.

Meiosis, polarization, kinetochore movements, and bivalent structure in, of male mantids, 265.

Melanoplus differentialis, development of tyrosinase in the parthenogenetic egg of, 157.

Microscope, electron, spermatozoon and fertilization membrane of Arbacia punctulata, as shown by, 151.

NEURULATION, in mechanically and chemically inhibited Amblystoma, 103.

OYSTERS, Polydora in, suspended in water, 69.

PARAMECIUM aurelia, sexual isolation, mating types and sexual responses to diverse conditions in variety 4, 36.

Peromyscus, species differences in rates of osmotic hemolysis in, 52.

PIERCE, E. LOWE. *See* Clarke, Pierce and Bumpus, 201.

Polarization, kinetochore movements, and bivalent structure in meiosis of male mantids, 265.

Polydora, in oysters suspended in water, 69.

Population, analysis of development of, in Daphnia at different temperatures, 116.

PRATT, DAVID M. Analysis of population development in Daphnia at different temperatures, 116.

PYLE, ROBERT W. The histogenesis and cyclic phenomena of the sinus gland and x-organ in crustacea, 87.

RADIOSENSITIVITY, of Arbacia eggs, in various salt solutions, 193.

RECKNAGEL, RICHARD O. *See* Wilbur and Recknagel, 193.

Reproduction, of Sagitta elegans, on Georges Bank, in relation to hydrographical conditions, 201.

RIS, HANS. A quantitative study of anaphase movement in the aphid Tamalia, 164.

SAGITTA elegans, distribution and reproduction of, on Georges Bank, 201.

Sea water, rate of breaking and size of halves of Arbacia punctulata egg, when centrifuged in hypo- and hypertonic, 141.

Segregation, seasonal, and hybridization in two races of a butterfly, 44.

Sinus gland, histogenesis and cyclic phenomena of, in crustacea, 87.

SONNEBORN, T. M., AND RUTH V. DIPPELL. Sexual isolation, mating types and sexual responses to diverse conditions in variety 4, Paramecium aurelia, 36.

Spermatozoon, of Arbacia punctulata, as shown by the electron microscope, 151.

Structure, bivalent, in the meiosis of male mantids, 265.

STUNKARD, HORACE W. The morphology and life history of the digenetic trematode, Zoögonoides laevis Linton, 1940, 227.

TAHMISIAN, THEODORE NEWTON. *See* Bodine and Tahmisian, 157.

Tamalia, a quantitative study of the anaphase movement in, 164.

Temperature, effect of, on population development of Daphnia, 116.

THIVY, FRANCESCA. New records of some marine Chaetophoraceae and Chaetosphaeridiaceae for North America, 244.

Trematode, digenetic, life history and morphology of, 227.

Tyrosinase, the development of, in the parthenogenetic egg of the grasshopper, 157.

VENUS mercenaria, action of acetylcholine on isolated heart of, 79.

WAIT, ROBERT B. The action of acetylcholine on the isolated heart of Venus mercenaria, 79.

WHITING, P. W. Intersexual females and intersexuality in Habrobracon, 238.

WILBUR, KARL M., AND RICHARD O. RECKNAGEL. The radiosensitivity of eggs of Arbacia punctulata in various salt solutions, 193.

X-ORGAN, histogenesis and cyclic phenomena of, in crustacea, 87.

ZOÖGONOIDES laevis Linton, life history and morphology of, 227.

Volume 85 Number 1

THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University

H. B. STEINBACH, Washington University
Managing Editor

AUGUST, 1943

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

SERIAL LIST

A SERIAL list of the holdings of The Marine Biological Laboratory was published as a separately bound supplement to the February issue of The Biological Bulletin. This supplement, covering approximately 80 pages, lists with cross references the 2258 titles of journals in the Library. Titles are listed alphabetically to conform to the arrangement of the stacks in the Library, and hence should serve as a guide book to the Library itself, as well as an aid in securing microfilm copies of articles. A few extra copies are still available. Orders may be directed to The Marine Biological Laboratory.

MICROFILM SERVICE

THE Library of The Marine Biological Laboratory is now prepared to supply microfilms of material from periodicals included in its extensive list. Through the generosity of Dr. Athertone Seidell, the essential equipment has been set up and put into operation. The Staff of The Marine Biological Laboratory Library is anxious to extend the Microfilm Service, particularly at this time when distance makes the Library somewhat inaccessible to many who normally use it. Investigators who wish films should send to the Librarian the name of the author of the paper, its title, and the name of the periodical in which it is printed, together with the volume and year of publication. The rates are as follows: $.30 for papers up to 25 pages, and $.10 for each additional 10 pages or fraction thereof. It is hoped that many investigators will avail themselves of this service.

Your Biological News

You would not go to the library to read the daily newspaper—probably you have it delivered at your home to be read at your leisure. Why, then, depend upon your library for your biological news?

Biological Abstracts is news nowadays. Abridgments of all the important biological literature are published promptly—in many cases before the original articles are available in this country. Only by having your own copy of *Biological Abstracts* to read regularly can you be sure that you are missing none of the literature of particular interest to you. An abstract of one article alone, which otherwise you would not have seen, might far more than compensate you for the subscription price.

Biological Abstracts is now published in six low priced sections, as well as the complete edition, so that the biological literature may be available to all individual biologists. Write for full information and ask for a copy of the section covering your field.

BIOLOGICAL ABSTRACTS
University of Pennsylvania
Philadelphia, Pa.

LANCASTER PRESS, Inc.

LANCASTER, PA.

⁂

THE EXPERIENCE we have gained from printing some sixty educational publications has fitted us to meet the standards of customers who demand the best.

We shall be happy to have workers at the MARINE BIOLOGICAL LABORATORY write for estimates on journals or monographs. Our prices are moderate.

INSTRUCTIONS TO AUTHORS

The Biological Bulletin accepts papers on a variety of subjects of biological interest. In general, a paper will appear within three months of the date of its acceptance. The Editorial Board requests that manuscripts conform to the requirements set below.

Manuscripts. Manuscripts should be typed in double or triple spacing on one side of paper, 8½ by 11 inches.

Tables should be typewritten on separate sheets and placed in correct sequence in the text. Explanations of figures should be typed on a separate sheet and placed at the end of the text. Footnotes, numbered consecutively, may be placed on a separate sheet at the end of the paper.

A condensed title or running page head of not more than thirty-five letters should be included.

Manuscripts must be returned to the Editor with the galley proof. Page proofs will be sent only on request.

Figures. The dimensions of the printed page, 5 by 7⅜ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. Explanatory matter should be included in legends as far as possible, not lettered on the illustrations. Figures should be prepared for reproduction as line cuts or halftones; other methods will be used only at the author's expense. Figures to be reproduced as line cuts should be drawn in black ink on white paper or blue-lined co-ordinate paper; those to be reproduced as halftones should be mounted on Bristol board and any designating letters or numbers should be made directly on the figures. The author's name should appear on the reverse side of all figures.

Literature cited. The list of literature cited should conform to the style set in this issue of The Biological Bulletin. Papers referred to in the manuscript should be listed on separate pages headed "Literature Cited." Where there are several papers cited, by the same author, the author's name should be repeated in each case.

Mailing. Manuscripts should be packed flat, not folded or rolled. Large charts and graphs may be rolled in a mailing tube.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost; approximate figures will be furnished upon request.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $1.75. Subscription per volume (three issues), $4.50.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Massachusetts, between July 1 and October 1, and to the Department of Zoology, Washington University, St. Louis, Missouri, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

BIOLOGY MATERIALS

The Supply Department of the Marine Biological Laboratory has a complete stock of excellent plain preserved and injected materials, and would be pleased to quote prices on school needs.

* * *

PRESERVED SPECIMENS

for

Zoology, Botany, Embryology,
and Comparative Anatomy

LIVING SPECIMENS

for

Zoology and Botany

including Protozoan and Drosophila Cultures, and Animals for Experimental and Laboratory Use.

MICROSCOPE SLIDES

for

Zoology, Botany, Embryology, Histology, Bacteriology, and Parasitology.

CATALOGUES SENT ON REQUEST

* * *

Supply Department
**MARINE
BIOLOGICAL LABORATORY**
Woods Hole, Massachusetts

CONTENTS

Page

Annual Report of the Marine Biological Laboratory ... 1

Sonneborn, T. M., and Ruth V. Dippell

Sexual Isolation, Mating Types, and Sexual Responses to Diverse Conditions in Variety 4, Paramecium Aurelia 36

Hovanitz, William

Hybridization and Seasonal Segregation in Two Races of a Butterfly Occurring Together in Two Localities 44

Levine, Harry P.

Species Differences in Rates of Osmotic Hemolysis Within the Genus Peromyscus 52

Lawson, Chester A.

Germarial Differences and the Production of Aphid Types .. 60

Loosanoff, Victor L., and James B. Engle

Polydora in Oysters Suspended in Water 69

Wait, Robert B.

The Action of Acetylcholine on the Isolated Heart of Venus Mercenaria 79

Volume 85 Number 2

THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University

H. B. STEINBACH, Washington University
Managing Editor

OCTOBER, 1943

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

SERIAL LIST

A SERIAL list of the holdings of The Marine Biological Laboratory was published as a separately bound supplement to the February issue of The Biological Bulletin. This supplement, covering approximately 80 pages, lists with cross references the 2258 titles of journals in the Library. Titles are listed alphabetically to conform to the arrangement of the stacks in the Library, and hence should serve as a guide book to the Library itself, as well as an aid in securing microfilm copies of articles. A few extra copies are still available. Orders may be directed to The Marine Biological Laboratory.

MICROFILM SERVICE

THE Library of The Marine Biological Laboratory is now prepared to supply microfilms of material from periodicals included in its extensive list. Through the generosity of Dr. Athertone Seidell, the essential equipment has been set up and put into operation. The Staff of The Marine Biological Laboratory Library is anxious to extend the Microfilm Service, particularly at this time when distance makes the Library somewhat inaccessible to many who normally use it. Investigators who wish films should send to the Librarian the name of the author of the paper, its title, and the name of the periodical in which it is printed, together with the volume and year of publication. The rates are as follows: $.30 for papers up to 25 pages, and $.10 for each additional 10 pages or fraction thereof. It is hoped that many investigators will avail themselves of this service.

Your Biological News

You would not go to the library to read the daily newspaper—probably you have it delivered at your home to be read at your leisure. Why, then, depend upon your library for your biological news?

Biological Abstracts is news nowadays. Abridgments of all the important biological literature are published promptly—in many cases before the original articles are available in this country. Only by having your own copy of *Biological Abstracts* to read regularly can you be sure that you are missing none of the literature of particular interest to you. An abstract of one article alone, which otherwise you would not have seen, might far more than compensate you for the subscription price.

Biological Abstracts is now published in six low priced sections, as well as the complete edition, so that the biological literature may be available to all individual biologists. Write for full information and ask for a copy of the section covering your field.

BIOLOGICAL ABSTRACTS
University of Pennsylvania
Philadelphia, Pa.

LANCASTER PRESS, Inc.

LANCASTER, PA.

☆

THE EXPERIENCE we have gained from printing some sixty educational publications has fitted us to meet the standards of customers who demand the best.

We shall be happy to have workers at the MARINE BIOLOGICAL LABORATORY write for estimates on journals or monographs. Our prices are moderate.

INSTRUCTIONS TO AUTHORS

The Biological Bulletin accepts papers on a variety of subjects of biological interest. In general, a paper will appear within three months of the date of its acceptance. **The Editorial Board requests that manuscripts conform to the requirements set below.**

Manuscripts. Manuscripts should be typed in double or triple spacing on one side of paper, 8½ by 11 inches.

Tables should be typewritten on separate sheets and placed in correct sequence in the text. Explanations of figures should be typed on a separate sheet and placed at the end of the text. Footnotes, numbered consecutively, may be placed on a separate sheet at the end of the paper.

A condensed title or running page head of not more than thirty-five letters should be included.

Manuscripts must be returned to the Editor with the galley proof. Page proofs will be sent only on request.

Figures. The dimensions of the printed page, 5 by 7⅜ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. **Explanatory matter should be included in legends as far as possible, not lettered on the illustrations. Figures should be prepared for reproduction as line cuts or halftones; other methods will be used only at the author's expense.** Figures to be reproduced as line cuts should be drawn in black ink on white paper or blue-lined co-ordinate paper; those to be reproduced as halftones should be mounted on Bristol board and any designating letters or numbers should be made directly on the figures. The author's name should appear on the reverse side of all figures.

Literature cited. **The list of literature cited should conform to the style set in this issue of The Biological Bulletin. Papers referred to in the manuscript should be listed on separate pages headed "Literature Cited." Where there are several papers cited, by the same author, the author's name should be repeated in each case.**

Mailing. Manuscripts should be packed flat, not folded or rolled. Large charts and graphs may be rolled in a mailing tube.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost; approximate figures will be furnished upon request.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $1.75. Subscription per volume (three issues), $4.50.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Massachusetts, between July 1 and October 1, and to the Department of Zoology, Washington University, St. Louis, Missouri, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

BIOLOGY MATERIALS

The Supply Department of the Marine Biological Laboratory has a complete stock of excellent plain preserved and injected materials, and would be pleased to quote prices on school needs.

PRESERVED SPECIMENS

for

Zoology, Botany, Embryology, and Comparative Anatomy

LIVING SPECIMENS

for

Zoology and Botany

including Protozoan and Drosophila Cultures, and Animals for Experimental and Laboratory Use.

MICROSCOPE SLIDES

for

Zoology, Botany, Embryology, Histology, Bacteriology, and Parasitology.

CATALOGUES SENT ON REQUEST

* * *

Supply Department

MARINE
BIOLOGICAL LABORATORY
Woods Hole, Massachusetts

CONTENTS

Page

PYLE, ROBERT W.

The Histogenesis and Cyclic Phenomena of the Sinus Gland and X-Organ in Crustacea 87

BURT, AGNES SANXAY

Neurulation in Mechanically and Chemically Inhibited Amblystoma 103

PRATT, DAVID M.

Analysis of Population Development in Daphnia at Different Temperatures 116

HARVEY, ETHEL BROWNE

Rate of Breaking and Size of the "Halves" of the Arbacia Punctulata Egg when Centrifuged in Hypo- and Hypertonic Sea Water 141

HARVEY, ETHEL BROWNE, AND THOMAS F. ANDERSON

The Spermatozoon and Fertilization Membrane of Arbacia Punctulata as Shown by the Electron Microscope 151

BODINE, JOSEPH HALL, AND THEODORE NEWTON TAHMISIAN

The Development of an Enzyme (Tyrosinase) in the Parthenogenetic Egg of the Grasshopper, Melanoplus Differentialis 157

RIS, HANS

A Quantitative Study of Anaphase Movement in the Aphid Tamalia 164

Volume 85 Number 3

THE

BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University

H. B. STEINBACH, Washington University
Managing Editor

DECEMBER, 1943

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

SERIAL LIST

A SERIAL list of the holdings of The Marine Biological Laboratory was published as a separately bound supplement to the February issue of The Biological Bulletin. This supplement, covering approximately 80 pages, lists with cross references the 2258 titles of journals in the Library. Titles are listed alphabetically to conform to the arrangement of the stacks in the Library, and hence should serve as a guide book to the Library itself, as well as an aid in securing microfilm copies of articles. A few extra copies are still available. Orders may be directed to The Marine Biological Laboratory.

MICROFILM SERVICE

THE Library of The Marine Biological Laboratory is now prepared to supply microfilms of material from periodicals included in its extensive list. Through the generosity of Dr. Athertone Seidell, the essential equipment has been set up and put into operation. The Staff of The Marine Biological Laboratory Library is anxious to extend the Microfilm Service, particularly at this time when distance makes the Library somewhat inaccessible to many who normally use it. Investigators who wish films should send to the Librarian the name of the author of the paper, its title, and the name of the periodical in which it is printed, together with the volume and year of publication. The rates are as follows: $.30 for papers up to 25 pages, and $.10 for each additional 10 pages or fraction thereof. It is hoped that many investigators will avail themselves of this service.

Your Biological News

You would not go to the library to read the daily newspaper—probably you have it delivered at your home to be read at your leisure. Why, then, depend upon your library for your biological news?

Biological Abstracts is news nowadays. Abridgments of all the important biological literature are published promptly—in many cases before the original articles are available in this country. Only by having your own copy of *Biological Abstracts* to read regularly can you be sure that you are missing none of the literature of particular interest to you. An abstract of one article alone, which otherwise you would not have seen, might far more than compensate you for the subscription price.

Biological Abstracts is now published in six low priced sections, as well as the complete edition, so that the biological literature may be available to all individual biologists. Write for full information and ask for a copy of the section covering your field.

BIOLOGICAL ABSTRACTS
University of Pennsylvania
Philadelphia, Pa.

LANCASTER PRESS, Inc.

LANCASTER, PA.

* * *

THE EXPERIENCE we have gained from printing some sixty educational publications has fitted us to meet the standards of customers who demand the best.

We shall be happy to have workers at the MARINE BIOLOGICAL LABORATORY write for estimates on journals or monographs. Our prices are moderate.

INSTRUCTIONS TO AUTHORS

The Biological Bulletin accepts papers on a variety of subjects of biological interest. In general, a paper will appear within three months of the date of its acceptance. **The Editorial Board requests that manuscripts conform to the requirements set below.**

Manuscripts. Manuscripts should be typed in double or triple spacing on one side of paper, 8½ by 11 inches.

Tables should be typewritten on separate sheets and placed in correct sequence in the text. Explanations of figures should be typed on a separate sheet and placed at the end of the text. Footnotes, numbered consecutively, may be placed on a separate sheet at the end of the paper.

A condensed title or running page head of not more than thirty-five letters should be included.

Manuscripts must be returned to the Editor with the galley proof. Page proofs will be sent only on request.

Figures. The dimensions of the printed page, 5 by 7⅜ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. **Explanatory matter should be included in legends as far as possible, not lettered on the illustrations. Figures should be prepared for reproduction as line cuts or halftones; other methods will be used only at the author's expense.** Figures to be reproduced as line cuts should be drawn in black ink on white paper or blue-lined co-ordinate paper; those to be reproduced as halftones should be mounted on Bristol board and any designating letters or numbers should be made directly on the figures. The author's name should appear on the reverse side of all figures.

Literature cited. **The list of literature cited should conform to the style set in this issue of The Biological Bulletin. Papers referred to in the manuscript should be listed on separate pages headed "Literature Cited." Where there are several papers cited, by the same author, the author's name should be repeated in each case.**

Mailing. Manuscripts should be packed flat, not folded or rolled. Large charts and graphs may be rolled in a mailing tube.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost; approximate figures will be furnished upon request.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $1.75. Subscription per volume (three issues), $4.50.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Massachusetts, between July 1 and October 1, and to the Department of Zoology, Washington University, St. Louis, Missouri, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster. Pa., under the Act of August 24, 1912.

BIOLOGY MATERIALS

The Supply Department of the Marine Biological Laboratory has a complete stock of excellent plain preserved and injected materials, and would be pleased to quote prices on school needs.

PRESERVED SPECIMENS

for

Zoology, Botany, Embryology,
and Comparative Anatomy

LIVING SPECIMENS

for

Zoology and Botany

including Protozoan and Drosophila Cultures, and Animals for Experimental and Laboratory Use.

MICROSCOPE SLIDES

for

Zoology, Botany, Embryology, Histology, Bacteriology, and Parasitology.

CATALOGUES SENT ON REQUEST

* * *

Supply Department
MARINE
BIOLOGICAL LABORATORY
Woods Hole, Massachusetts

CONTENTS

Page

HARRIS, DANIEL L.

The Osmotic Properties of Cytoplasmic Granules of the Sea Urchin Egg 179

WILBUR, KARL M., AND RICHARD O. RECKNAGEL

The Radiosensitivity of Eggs of Arbacia Punctulata in Various Salt Solutions 193

CLARKE, GEORGE L., E. LOWE PIERCE AND DEAN F. BUMPUS

The Distribution and Reproduction of Sagitta Elegans on Georges Bank in Relation to the Hydrographical Conditions. 201

STUNKARD, HORACE W.

The Morphology and Life History of the Digenetic Trematode, Zoögonoides Laevis Linton, 1940 227

WHITING, P. W.

Intersexual Females and Intersexuality in Habrobracon 238

THIVY, FRANCESCA

New Records of Some Marine Chaetophoraceae and Chaetosphaeridiaceae for North America 244

HUGHES-SCHRADER, SALLY

Polarization, Kinetochore Movements, and Bivalent Structure in the Meiosis of Male Mantids 265

MBL/WHOI LIBRARY
WH 17J4 .